



EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
02.11.2016 Bulletin 2016/44

(51) Int Cl.:
C07K 14/775 ^(2006.01) **C07K 19/00** ^(2006.01)
A61K 38/17 ^(2006.01) **C07K 14/195** ^(2006.01)

(21) Application number: **11702831.6**

(86) International application number:
PCT/EP2011/051761

(22) Date of filing: **07.02.2011**

(87) International publication number:
WO 2011/095628 (11.08.2011 Gazette 2011/32)

(54) **FUSION PROTEINS COMPRISING ANTIGENIC APOB-100 PROTEIN FRAGMENTS AND A PROTEIN CARRIER, RELATED COMPOSITIONS, METHODS AND SYSTEMS FOR TREATMENT AND/OR PREVENTION OF ATHEROSCLEROSIS**

FUSIONSPROTEINE MIT ANTIGENEN APOB-100-PROTEINFRAGMENTEN UND EINEM PROTEINTRÄGER SOWIE ENTSPRECHENDE ZUSAMMENSETZUNGEN, VERFAHREN UND SYSTEME ZUR BEHANDLUNG UND/ODER VERHINDERUNG VON ARTERIOSKLEROSE

PROTÉINES DE FUSION COMPRENANT DES FRAGMENTS DE PROTÉINE APOB-100 ANTIGÉNIQUES ET UN SUPPORT DE PROTÉINE, COMPOSITIONS ASSOCIÉES, PROCÉDÉS ET SYSTÈMES POUR LE TRAITEMENT ET/OU LA PRÉVENTION DE L'ATHÉROSCLÉROSE

(84) Designated Contracting States:
**AL AT BE BG CH CY CZ DE DK EE ES FI FR GB
GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO
PL PT RO RS SE SI SK SM TR**

(30) Priority: **05.02.2010 US 201061302051 P**
04.02.2011 US 201113021635

(43) Date of publication of application:
12.12.2012 Bulletin 2012/50

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DescriptionTechnical field

5 **[0001]** The present disclosure relates to fusion proteins and related compositions, methods and systems for treatment and/or prevention of atherosclerosis.

Background

10 **[0002]** Atherosclerosis is currently viewed as a chronic lipid-related and immune-mediated inflammatory disease of the arterial walls. Many immune components have been identified that participate in atherogenesis and pre-clinical studies have yielded promising results suggesting that immuno-modulatory therapies targeting these components can reduce atherosclerosis.

15 Summary

[0003] Described herein, are methods and systems for inducing immunomodulatory responses in an individual. In several instances, the immunomodulatory responses induced by the methods and systems of the present disclosure are associated to a therapeutic or preventive effect related to atherosclerosis in the individual or a condition associated thereto.

20 **[0004]** Accordingly, in one aspect, the invention provides a fusion protein comprising

an antigenic fragment of apoB-100 protein, or a derivative thereof, and
subunit B of cholera toxin as a protein carrier,

25 wherein the antigenic fragment of apoB-100 or derivative thereof and the protein carrier are linked in the fusion protein in a 1:1 fragment:carrier molar ratio; and

wherein the fusion protein is capable of inducing antigen specific regulatory T cells, the antigen specific regulatory T cells being specific for the antigenic fragment of apoB- 100 or derivative thereof,

30 for use to treat and/or prevent atherosclerosis in an individual,

wherein the fusion protein is for mucosal administration to the individual in an amount effective to elicit an antigen specific Treg immunomodulatory response specific for the antigenic fragment of apoB-100 protein, or derivative thereof, in the individual,

35 and wherein a derivative of an antigenic fragment of apoB-100 protein indicates a polypeptide structurally related to the antigenic fragment of apoB-100 and derivable from the antigenic fragment of apoB-100 by a modification that introduces a feature that is not present in the antigenic fragment of apoB-100, while retaining one or more antigenic properties of apoB-100 or the antigenic fragment of apoB-100.

[0005] In one aspect, the antigenic fragment of apoB-100 may comprise one or more peptide selected from the group consisting of P2, P45, P102 and P210.

40 **[0006]** The invention also provides an immunogenic composition comprising the fusion protein for use according to the invention together with an adjuvant and/or a pharmaceutically acceptable excipient.

[0007] The invention also provides a method to produce a fusion protein, the method comprising

45 attaching an antigenic fragment of apoB-100, or a derivative thereof, to a protein carrier in a 1:1 fragment:carrier molar ratio to provide a fusion protein capable of inducing antigen specific regulatory T cells, wherein the protein carrier is subunit B of cholera toxin,

the antigen specific regulatory T cells being specific for the fragment of apoB-100 or the derivative thereof,

50 wherein a derivative of an antigenic fragment of apoB-100 protein indicates a polypeptide structurally related to the antigenic fragment of apoB-100 and derivable from the antigenic fragment of apoB-100 by a modification that introduces a feature that is not present in the antigenic fragment of apoB-100, while retaining one or more antigenic properties of apoB-100 or the antigenic fragment of apoB-100.

[0008] In one aspect, the antigenic fragment of apoB-100 may comprise one or more of P2, P45, P102, and P210.

55 **[0009]** The invention also provides an *in vitro* method to induce an antigen specific Treg cell, the method comprising

contacting a Treg cell with the fusion protein for use according to the invention, the contacting performed for a time and under conditions to allow induction of a Treg response,

wherein the contacting results in the induction of an antigen-specific immune response specific for the fragment of

apoB-100, or derivative thereof, comprised in the fusion protein.

[0010] Described herein is a fusion protein. The fusion protein comprises an antigenic fragment of apoB-100 protein or a derivative thereof and a protein carrier, the antigenic fragment and the carrier comprised in the fusion protein in a fragment:carrier 1:1 molar ratio the fusion protein capable of inducing antigen specific regulatory T cells, the antigen specific regulatory T cells specific for the antigenic fragment of apoB-100.

[0011] Also described is a method to treat and/or prevent atherosclerosis in an individual. The method comprises administering to the individual an effective amount of a fusion protein herein described, the effective amount eliciting an antigen specific Treg immunomodulatory response in the individual, the antigen specific Treg immunomodulatory response specific for the antigenic fragments of apoB-100 or a derivative thereof.

[0012] Also described is a composition. The composition comprises a fusion protein herein described and an adjuvant and/or excipient. In several instances the adjuvant and/or excipients are pharmaceutically acceptable and the composition is pharmaceutical composition

[0013] Also described is a method to produce a fusion protein. The method comprises conjugating a fragment of apoB-100 or a derivative thereof with a suitable protein carrier to provide a fusion protein capable of inducing antigen specific regulatory T cells, the antigen specific regulatory T cells specific for the fragment of apoB-100 or the derivative thereof

[0014] Also described is a method to induce an antigen specific Tregulatory cell. The method comprises contacting a Tregulatory cell with a fusion protein herein described for a time and under condition to allow induction of a Tregulatory response, wherein the contacting results in an antigen-specific induction of a Tregulatory cell that is specific for the fragment of apoB-100 or derivative thereof comprised in the fusion protein.

[0015] The methods and systems herein described can be used in connection with applications wherein reduction of plaque, attenuation of plaque growth and/or a therapeutic or preventive effect for atherosclerosis in an individual is desired.

[0016] The details of one or more embodiments of the disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

Brief description of the drawings

[0017] The accompanying drawings, which are incorporated into and constitute a part of this specification, illustrate one or more embodiments of the present disclosure and, together with the detailed description and examples sections, serve to explain the principles and implementations of the disclosure.

Figure 1 shows data concerning intranasal p210-CTB administered twice weekly for 12 weeks reduced atherosclerotic lesion size in the aortic root of female *apoe*^{-/-} mice. **(A)** Data from the three groups are shown: black circles represent animals from the p210-CTB group, dotted circles animals from the OVA-CTB group and white circles animals from the control group, respectively. * indicates $p < 0.05$. **(B-D)** Representative photomicrographs show oil red O stained aortic root sections from each group (50X magnification). **(E)** Nasal immunization with CTB fusion proteins increased FoxP3 and IL-10 mRNA levels in thoracic aorta from *apoe*^{-/-} mice after 12 weeks of treatment. mRNA transcript ratios based on HPRT expression are shown for each gene of interest for all three groups. * indicates $p < 0.05$

Figure 2 shows data nasal immunization induced a systemic humoral immune response in *apoe*^{-/-} mice. **(A)** IgG-anti-p210 titers in mouse plasma; titration curves are shown in the inset. **(B)** p210-specific IgM titers in plasma from the same mice. * indicates $p < 0.05$.

Figure 3 shows data concerning nasal immunization altered T cell subset composition in lung mucosa. Flow cytometric analysis of intracellular subset markers, with cytokine-producing CD4⁺ T cells as percentage of total CD4⁺ T cells for each of the three groups. **(A)** Interferon- γ ; **(B)** IL-17; **(C)** IL-4; **(D)** FoxP3. *) indicates $p < 0.05$.

Figure 4 shows data concerning nasal p210-CTB immunization induced IL-10-producing CD4⁺ T cells and apoB-100-specific Treg activity in spleen. **(A)** Flow cytometric analysis of cultured spleen cells stained for intracellular IL-10. **(B)** Representative flow cytometric plots. **(C)** Splenic effector cells at 2.5×10^5 cells/well were generated from *apoe*^{-/-} mice that had been immunized with human apoB 100. The stimulation index represents the ratio of ³H thymidine uptake upon stimulation with human apo100 (20 μ g/mL) relative to unstimulated cells. Proliferation of effector cells alone is indicated in the leftmost bar of each group. Addition of purified CD4⁺ T cells from nasally immunized animals is indicated at different ratios to effector cells. * $p < 0.05$.

Figure 5 shows data concerning a protective effect of nasal p210-CTB immunization on atherosclerotic lesion size

does not depend on TGF- β signaling in T cells. **A)** Lesion size in the aortic root of *apoe*^{-/-} x CD4dnTGF β RIItg mice immunized with p210-CTB (black dots) or OVA-CTB (grey dots). Effect of immunization on p210-specific antibody titers of IgG class **(B)** and IgM **(C)**. * $p < 0.05$.

Figure 6 shows data concerning lesion size in immunized mice. Fractional area of lesions in aortic root of *apoe*^{-/-} mice treated nasally with p210-CTB (black bar), OVA-CTB (dotted bar) and untreated controls (white bar). Mean $\pm$ SD values are shown. * indicates $p < 0.05$.

Figure 7 shows data concerning plasma lipoprotein profiles analyzed by FPLC. Thick lines represent immunized groups: black = p210-CTB, grey = OVA-CTB and white shows the control group. Thin lines represent SEM. CR/VLDL = chylomicrons/very low density lipoproteins; LDL = low density lipoproteins; HDL = high density lipoproteins.

Figure 8 shows data concerning T cells in lesions in immunized mice, and in particular representative immunohistochemical stains of atherosclerotic lesion in the aortic root of *apoe*^{-/-} mice. Arrows indicate FoxP3⁺ cells; they were CD4⁺ in directly adjacent sections.

Figure 9 shows data concerning serum IgG1 antibody titers to apoB-100 peptide. ELISA analysis of sera from *apoe*^{-/-} mice treated with p210-CTB (black bar), OVA-CTB (dotted bar) or controls (white bar). * indicates $p < 0.05$.

Figure 10 shows data concerning serum IgG2a antibody titers to apoB-100 peptide. *Apoe*^{-/-} mice treated with p210-CTB (black bar), OVA-CTB (dotted bar) or controls (white bar). * indicates $p < 0.05$.

Figure 11 shows data concerning total IgG in sera of immunized mice. *Apoe*^{-/-} mice treated with p210-CTB (black bar), OVA-CTB (dotted bar) or controls (white bar).

Figure 12 shows data concerning total IgM in sera of immunized mice. *Apoe*^{-/-} mice treated with p210-CTB (black bar), OVA-CTB (dotted bar) or controls (white bar).

Figure 13 shows data concerning T cell subsets in spleen after immunization. Flow cytometric analysis of intracellular cytokine expression is shown as percentage of cytokine-producing cells per CD4⁺ T cells for each of the three groups. **(A)** Interferon- γ ; **(B)** IL-17; **(C)** IL-4; **(D)** FoxP3.

Figure 14 shows data concerning abrogation of Treg suppressor effect upon separation of cells. Cocultures of effector cells and CD4⁺ T cells from nasally immunized mice are indicated by bars (generated from dark blotted = p210-CTB, light dotted = OVA-CTB and white = control group animals). Abrogated effect of CD4⁺ T cell-mediated inhibition of proliferation when effector cells and CD4⁺ T cells are cultured separate from each other in a transwell plate (contact inhibition assay).

Figure 15 shows data concerning cytokine levels for TGF- β and IL-10 in the supernatants from the coculture assay. Striped bars represent effector cells alone. Cocultures of effector cells and CD4⁺ T cells from nasally immunized mice are indicated by plain bars (generated from dark dotted = p210-CTB, light dotted = OVA-CTB and white = control group animals).

Figure 16 shows data concerning lesion size in immunized mice with defective TGF β receptors on T cells. Fractional area of the lesion in the aortic root of *apoe*^{-/-} x CD4dnTGF β RIItg mice treated nasally with p210-CTB (black bar) or OVA-CTB (dotted bar). Mean $\pm$ SD values are shown. * indicates $p < 0.05$.

Figure 17 shows data concerning mRNA levels in aorta of mice with defective TGF β receptors on T cells. Real-time reverse-transcription PCR normalized to HPRT in thoracic aorta of *apoe*^{-/-} x CD4dnTGF β RIItg mice. Of note, the development of functional Foxp3⁺ Treg cells depends on TGF β . Therefore, detailed information concerning the relevance of FoxP3-expressing cells in *apoe*^{-/-} x CD4dnTGF β RIItg mice require further investigation.

Figure 18 shows data concerning IgG antibodies to mouse LDL in mice immunized with LDL or OVA. C57BL/6J mice were immunized with mouse LDL (mLDL) or ovalbumin (OVA). ELISA was performed with serum dilutions in wells coated with mouse LDL, followed by alkaline phosphatase-labeled anti-mouse-IgG. SC, subcutaneous administration, IN, intranasal administration.

Detailed description

[0018] Described herein are fusion proteins, compositions methods and systems that in several instances are suitable to be used for immunoprotection against atherosclerosis.

[0019] The term "fusion protein" as used herein indicates a protein created through the attaching of two or more polypeptides which originated from separate proteins. In particular fusion proteins can be created by recombinant DNA technology and are typically used in biological research or therapeutics. Fusion proteins can also be created through chemical covalent conjugation with or without a linker between the polypeptides portion of the fusion proteins.

[0020] The term "attach" or "attached" as used herein, refers to connecting or uniting by a bond, link, force or tie in order to keep two or more components together, which encompasses either direct or indirect attachment such that for example where a first polypeptide is directly bound to a second polypeptide or material, and the embodiments wherein one or more intermediate compounds, and in particular polypeptides, are disposed between the first polypeptide and the second polypeptide or material.

[0021] The term "protein" or "polypeptide" as used herein indicates an organic polymer composed of two or more amino acid monomers and/or analogs thereof. The term "polypeptide" includes amino acid polymers of any length including full length proteins and peptides, as well as analogs and fragments thereof. A polypeptide of three or more amino acids is also called an oligopeptide. As used herein the term "amino acid", "amino acidic monomer", or "amino acid residue" refers to any of the twenty naturally occurring amino acids including synthetic amino acids with unnatural side chains and including both D and L optical isomers. The term "amino acid analog" refers to an amino acid in which one or more individual atoms have been replaced, either with a different atom, isotope, or with a different functional group but is otherwise identical to its natural amino acid analog.

[0022] In the particular in several embodiments, fusion proteins, compositions methods and systems are described that in several embodiments are suitable for eliciting an antigen specific T regulatory cells response in an individual.

[0023] The term "antigen", as it is used herein, relates to any substance that, when introduced into the body can stimulate an immune response. Antigens comprise exogenous antigens (antigens that have entered the body from the outside, for example by inhalation, ingestion, or injection) and endogenous antigens or autoantigens (antigens that have been generated within the body). In particular, an "autoantigen" is an antigen that despite being a normal tissue constituent is the target of a humoral or cell-mediated immune response. Exemplary autoantigens comprise autoantigens associated to atherogenesis and/or atherosclerosis provided by low-density lipoprotein and its constituent protein, ApoB 100.

[0024] The term "regulatory T cell" or "T reg" as used herein indicates a component of the immune system that suppress immune responses of other cells, and comprise T cells that express the CD8 transmembrane glycoprotein (CD8+ T cells); T cells that express CD4, CD25, and Foxp3 (CD4+CD25+ regulatory T cells); and other T cell types that have suppressive function identifiable by a skilled person. T reg comprise both naturally occurring T cells and T cells generated in vitro.

[0025] The term "antigen-specific" as used indicates an immunitary response, and in particular, immunological tolerance, for a certain antigen which is characterized by a substantially less or no immune response (and in particular, immunological tolerance) for another antigen. Accordingly, an antigen specific regulatory T cell, specific for one or more autoantigens is able, under appropriate conditions to minimize to the specific immune response to the one or more autoantigens with substantially less or no minimizing effect on the immune response towards other antigens or autoantigens.

[0026] Fusion proteins comprising autoantigen associated to atherogenesis and/or atherosclerosis and related methods and systems are herein described that are capable of eliciting an autoantigen specific Treg response and that in several instances can be used for treating and/or preventing atherosclerosis or a condition associated thereto in an individual.

[0027] The term "atherosclerosis" as used herein indicates an inflammatory condition, and in particular the condition in which an artery wall thickens as the result of a build-up of fatty materials such as cholesterol. In some cases, atherosclerosis is treated with statin therapy (1). In several cases, atherosclerosis is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, in large part due to the accumulation of macrophage white blood cells and promoted by Low-density lipoproteins (plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (HDL), (see apoA-1 Milano). Lipid retention and modification in the arterial intima in some cases elicit a chronic inflammatory process with autoimmune responses and the development of atherosclerotic lesions (2). Both adaptive and innate immune mechanisms have been described as contributors to this process (3-6). While pattern recognition receptors of innate immunity are believed to account for cholesterol uptake and contribute to activation of macrophages and endothelial cells, antigen-specific T cells recognizing low density lipoprotein (LDL) particles in the intima provide strong proinflammatory stimuli that accelerate atherogenesis. Atherosclerosis is commonly referred to as a hardening or furring of the arteries. It is believed to be caused by the formation of multiple plaques within the arteries. Typically, autoimmune responses to low-density lipoproteins (LDL) contribute to its progression, while immunization with LDL may induce

atheroprotective or proatherogenic responses.

[0028] Zhao Xiaoning et al., 2009, Circulation, 120(18), Suppl. 2, S1018, 82nd Scientific Session of the American-Heart-Association; Orlando, FL, USA; November 14 -18, 2009, discloses the fusion of ApoB100 P210 antigenic peptide (conjugated) to cBSA. These molecules are used combined with alum as adjuvant in vaccination of mice and demonstrated to enhance CD8+ regulatory T-cell response.

[0029] The term "treating" or "treatment" as used herein indicates any activity that is part of a medical care for, or that deals with, a condition medically or surgically. The term "preventing" or "prevention" as used herein indicates any activity, which reduces the burden of mortality or morbidity from a condition in an individual. This takes place at primary, secondary and tertiary prevention levels, wherein: a) primary prevention avoids the development of a disease; b) secondary prevention activities are aimed at early disease treatment, thereby increasing opportunities for interventions to prevent progression of the disease and emergence of symptoms; and c) tertiary prevention reduces the negative impact of an already established disease by restoring function and reducing disease-related complications.

[0030] The term "condition" as used herein indicates as usually the physical status of the body of an individual (as a whole or of one or more of its parts) that does not conform to a physical status of the individual (as a whole or of one or more of its parts) that is associated with a state of complete physical, mental and possibly social well-being. Conditions herein described include but are not limited to disorders and diseases wherein the term "disorder" indicates a condition of the living individual that is associated to a functional abnormality of the body or of any of its parts, and the term "disease" indicates a condition of the living individual that impairs normal functioning of the body or of any of its parts and is typically manifested by distinguishing signs and symptoms. Exemplary conditions include but are not limited to injuries, disabilities, disorders (including mental and physical disorders), syndromes, infections, deviant behaviors of the individual and atypical variations of structure and functions of the body of an individual or parts thereof.

[0031] The wording "associated to" as used herein with reference to two items indicates a relation between the two items such that the occurrence of a first item is accompanied by the occurrence of the second item, which includes but is not limited to a cause-effect relation and sign/symptoms-disease relation.

[0032] The term "individuals" as used herein indicates a single biological organism such as higher animals and in particular vertebrates such as mammals and more particularly human beings.

[0033] In several instances, induction of an antigen specific Tregulatory cell response is provided by a fusion protein comprising an antigenic fragment of apoB-100 and a protein carrier attached directly or through a linker in an fragment:carrier 1:1 proportion.

[0034] The term "fragment" as used herein indicates a portion of a polypeptide of any length. A skilled person will understand that the term encompasses peptides of any origin which have a sequence corresponding to the portion of the polypeptide at issue. An antigenic fragment of apoB 100 is accordingly a portion of apoB-100 that presents antigenic properties. Antigenic fragments of apoB-100 herein described also include possible derivatives thereof.

[0035] The term "derivative" as used herein with reference to a first polypeptide (e.g. apoB-100 antigenic fragment), indicates a second polypeptide that is structurally related to the first polypeptide and is derivable from the first polypeptide by a modification that introduces a feature that is not present in the first polypeptide, while retaining functional properties of the first polypeptide. Accordingly, a derivative polypeptide of an antigenic fragment of apoB-100, usually differs from the original polypeptide or portion thereof by modification of the amino acidic sequence that might or might not be associated with an additional function not present in the original polypeptide or portion thereof. A derivative polypeptide of an antigenic fragment of apoB-100 retains however antigenic properties comparable to the ones described in connection with apoB-100 or the antigenic fragment thereof. Retaining of one or more antigenic properties can be verified with methods identifiable by a skilled person upon reading of the present disclosure, on the basis of the specific antigenic property of the fragment at issue. Exemplary methods comprise immunizing an animal (e.g. mouse) with a candidate derivative, determining production of antibody specific for the derivative in the animal (e.g. by ELISA such as immunometric ELISA) and comparing the determined antibody production for the candidate derivative with a corresponding antibody production of the fragment. Additional methods to determine further antigenic properties can be identified by a skilled person upon reading of the present disclosure.

[0036] The term "protein carrier" as used herein indicates proteins that transport a specific substance or group of substances through intracellular compartments or in extracellular fluids (e.g. in the blood) or else across the cell membrane. Exemplary carrier proteins comprise subunit B of cholera toxin, Avidin, BTG protein, Bovine G globulin, Bovine Immunoglobulin G, Bovine Thyroglobulin, Bovine Serum Albumin (BSA), Conalbumin, Edestein, Exoprotein A from Pseudomonas aeruginosa, HC (Hemocyanin from crab Paralithodes camtschatica), Helix Promatia Haemocyanin (HPH), Human Serum Albumin (HSA), KTI (Kunits trypsin inhibitor from soybeans), Keyhole Limpet Haemocyanin (KLH), LPH (Haemocyanin from Limulus polyphemus) Ovalbumin, Pam3Cys-Th, Polylysine, porcine Thyroglobulin (PTG), Purified Protein Derivative (PPD), Rabbit Serum Albumin (RSA), Soybeab Trypsin Inhibitor (STI) (Sunflower Globulin (SFG) and additional molecules identifiable by a skilled person. Additional carriers comprise molecule having immunogenic activities including cytokines such as IL-10, IL-12, IL-4 IL-16 and Transforming Growth Factor Beta (TGFp).

[0037] In some instances, attachment of the carrier is performed at the C-terminus or N-terminus of the fragment. In

an instance the fusion protein can be provided as a single polypeptide through recombinant DNA technology and related processes, such as cloning, chimeric constructs, Polymerase Chain Reaction and additional procedures identifiable by a skilled person. In some instances, attachment can be performed through chemical linkage of the fragment to the carrier using methods also identifiable by a skilled person.

[0038] In some instances, the antigenic fragment of apoB-100 comprises amino acids 3136-3155 of human apoB-100 (p210) and/or additional fragments selected from the peptides illustrated in the Examples section.

[0039] In particular in some instances the fragment portion of the fusion product can comprise one or more of peptides P2, P11, P25, P32, P45, P74, P102, P129, P143, P148, P154, P162, P210, P219, P240. More particularly, in some instances the fragment portion of the fusion products can comprise one or more of peptides P2, P45, P102 and P210.

[0040] In an instance, wherein the fragment portion of the fusion protein comprise more than one peptide, the fragment portion can comprise up to 10 peptides in a construct that, at least in some of those instances, is expected to have effects analogous to those of cancer or infectious vaccines, such as the ones described in (35).

[0041] As skilled person will be able to identify suitable combination of peptides for a desired immunogenic, preventive and/or therapeutic effect upon reading of the present disclosure.

[0042] In some instances, the carrier protein can comprise at least one monomers of the subunit B of cholera toxin which can be formed by a recombinant pentameric B oligomer that is capable of binding GM-1 receptors (e.g. on the surface of intestinal epithelial cells). In particular, in some instances, the carrier protein can be formed by at least one of five identical monomers with a molecular weight of approximately 11.6 kDa recombinant pentameric B oligomer molecule. In some of those instances, the monomers are tightly linked into a trypsin-resistant pentameric ring-like structure with a molecular weight of approximately 58 kDa.

[0043] In some instances, the antigenic fragments can be attached to the carrier molecule using biological genetic engineering to produce a fusion protein (with single or multiple copies of the immunogenic peptide) and procedures identifiable by a skilled person upon reading of the present disclosure.

[0044] In some instances, the antigenic fragments can be attached to the carrier molecule using chemical covalent conjugation (with or without a linker group) and procedures identifiable by a skilled person upon reading of the present disclosure.

[0045] In some instances, fusion products or antigenic fragments can be used in the treatment of atherosclerosis and or for induction of regulatory T-cells

[0046] In some instances, antigen-specific immunomodulation by vaccination is an approach used to prevent or treat chronic inflammatory diseases associated to atherogenesis. In some of those cases, by mobilizing protective immune responses in an antigen-specific manner, side effects due to hampered host defense against infections can be avoided. Exemplary protocols comprise protocols described to treat atherosclerosis in mice and rabbits immunized with LDL, beta2-glycoprotein-Ib, or heat-shock protein 60/65, and parenteral (7-10) as well as oral (11-14) immunization reduced atherosclerotic disease in hyperlipidemic animals.

[0047] In some instances, antigen-specific immunoprotection can be achieved through several different mechanisms, such as production of protective antibodies, deletion or inactivation (anergy) of pathogenic T cell clones, or induction of suppressive cellular immunity mediated by the family of regulatory T cells (Treg) (15-16). In some of those instances, immunization with immunodominant peptide sequences can be performed in several cases in alternative to immunization with LDL particles (17-18).

[0048] In an instance, an immunization protocol that facilitates selective targeting of antigen-specific regulatory T cells can be performed. The type of immune response triggered is largely determined by the route of immunization.

[0049] In several instances, fusion products or antigenic fragments herein described can be administered to an individual using various routes of administration including subcutaneous, intramuscular, parenteral, and systemic and mucosal administration such as oral and/or nasal. In particular, the mucosal linings of airways and intestines contain lymphatic tissue that, when exposed to antigen, elicits anti-inflammatory, immunosuppressive responses (19). Distinct immunological features of the respiratory and intestinal mucosa lead to partly different types of protective immunity upon antigen exposure by the nasal or oral route (20). In some instances, the B subunit of cholera toxin (CTB) promotes uptake of antigen via the nasal and oral mucosa and induction of protective immunity (21, 22).

[0050] In some instances, administration of carrier/adjuvant/peptide vaccines is performed for a time and under condition to activate regulatory T cells and down-regulate pathogenic autoimmunity against Apo B.

[0051] In particular, in some instances, administration of a fusion protein is performed by nasal administration of an apoB 100 peptide-CTB fusion protein (p210-CTB). In some instances, treatment with p210-CTB significantly reduced atherosclerosis in apoE^{-/-} mice and was associated with induction of antigen-specific Treg activity.

[0052] In some instances, intranasal immunization with an apoB-100 fusion protein induces antigen-specific regulatory T cells and reduces atherosclerosis.

[0053] In several instances, nasal administration of an apoB 100 peptide fused to CTB attenuates atherosclerosis and induces regulatory Tr1 cells that inhibit T effector responses to apolipoprotein B-100.

[0054] In some instances, fusion products, compositions and/or methods compositions herein described can be used

a novel strategy for induction of atheroprotective immunity involving antigen-specific regulatory T cells. In particular, by nasal administration of a fusion protein between an immunodominant peptide of apoB-100 and immunomodulatory CTB, we were able to induce an atheroprotective immune response to apoB-100 that involved expansion of antigen-specific regulatory CD4⁺ T cells and inhibition of aortic lesion development.

[0055] In several instances, induction of antigen-specific Treg with fusion protein methods and systems herein described provides atheroprotection using parenteral or oral routes for LDL immunization. Additionally, results illustrated in the Examples section concerning induction of antigen-specific atheroprotective immunity mucosal immunization in *apoe*^{-/-} mice, which spontaneously develop atherosclerosis and are therefore already sensitized to plaque antigens such as LDL particles at the time of vaccination, supports the conclusion that a comparable approach in humans with pre-existing lesions is expected to provide immunization.

[0056] In some instances, fusion protein herein described fusion proteins triggers a mechanism of atheroprotection where the atheroprotective effect paralleled an induction of Treg suppression of apoB-100-specific effector T cells and an increase in IL-10⁺ CD4⁺ T cells. In particular, in some instances, nasal immunization with p210-CTB protects against atherosclerosis by induction of antigen-specific, IL-10⁺ regulatory Tr1 cells. A possible explanation that is provided herein for guidance purpose only and it is not intended to be limiting is that atheroprotection in several cases does not involve the immunosuppressive cytokine TGF- β since nasal immunization with p210-CTB reduced atherosclerosis also in mice lacking functional TGF- β receptors on T cells.

[0057] In some instances, fusion protein herein described provide an antigen-specific as well as antigen-independent effects similar to what reported in studies of Treg(25). In particular, in some instances, Treg suppress conventional effector T cells with the same antigen specificity. In some instances, Treg exert major effects on antigen-presenting cells in an antigen-independent manner. In some instances, the antigen-specific atheroprotection is paralleled by inhibition of apoB 100-specific effector T cells by Treg specific for p210 but not OVA. These findings support a protective role for autoantigen-specific Treg in atherosclerosis.

[0058] In some instances, two major types of Treg induced in the periphery by antigen exposure have been identified: FoxP3⁺ induced Treg (Th3) 14 and Tr1 cells (26). Tr1 cells are FoxP3 negative, secrete IL-10, and are believed to play an important role when regulatory immunity is induced by nasal immunization (27), (28). In some instances, where atheroprotection is induced by nasal immunization and associated with suppressor T cell activity and IL-10 producing CD4⁺ T cells, administration of fusion protein herein described is associated with Tr1 induction by p210-CTB. CD4⁺ T cells with antigen-specific suppressor activity were derived from spleen, a known reservoir of Tr1 cells(26).

[0059] In some instances, FoxP3⁺ Treg can contribute to atheroprotection in this model following administration of a fusion protein herein described as indicated by an increase of FoxP3 mRNA was increased in the aorta of nasally immunized mice. In some instances, these cells can not only act directly to control proinflammatory effector T cells but also promote the activation of Tr1 cells (19). In some instances, wherein abrogation of TGF- β signaling is detected, Tr1 cells do not extinguish atheroprotection.

[0060] In some instances, where Treg markers are elevated also in OVA-CTB immunized mice, antigenically nonspecific effects can synergize with antigen-specific ones to confer protection.

[0061] In some instances, antibodies to the apoB 100 peptide sequence are induced by nasal immunization, but do not crossreact with native mouse LDL particles. Furthermore, in some instances where particles antibody titers are not correlated with lesion size and no difference in lipoprotein profiles is detected between apoB-100-CTB immunized and OVA-CTB, immunized mice atheroprotection is associated to immunomodulation rather than antibody-dependent elimination of LDL.

[0062] In some instances, fusion proteins herein described are comprised in a composition together with suitable adjuvant and/or excipients.

[0063] The term adjuvant as used herein indicates a pharmacological or immunological agent that modify the effect of other agents (e.g., drugs, vaccines) while having few if any direct effects when given by themselves. They are often included in vaccines to enhance the recipient's immune response to a supplied antigen while keeping the injected foreign material at a minimum. Types of adjuvants include: Immunologic adjuvant that stimulate the immune system and increase the response to a vaccine, without having any specific antigenic effect in itself.

[0064] The term excipients as used herein indicates... an inactive substance used as a carrier for the active ingredients of a medication. Exemplary excipients can also be used to bulk up formulations that contain very potent active ingredients, to allow for convenient and accurate dosage. In addition to their use in the single-dosage quantity, excipients can be used in the manufacturing process to aid in the handling of the active substance concerned. Depending on the route of administration, and form of medication, different excipients may be used that are identifiable by a skilled person.

[0065] In some instances, the compositions comprises Selected (immunogenic) peptide fragments of apoB-100 (single or multiple copies) fused with a carrier molecule and possibly toxins/toxoids: tetanus toxin, diphtheria toxoid, B subunit of cholera toxin, as well as BSA, HAS, rHSA, KLH, ovalbumin

[0066] In some instances, the adjuvants and excipients are pharmaceutically acceptable and the resulting composition is a pharmaceutical composition. In some of those instances, the pharmaceutical composition is a vaccine.

[0067] In some instances, adjuvants are components of the vaccine formulation that enhance immunogenicity of the antigen, for instance by promoting their uptake by antigen-presenting cells (17, 29). Interestingly, two studies documented an atheroprotective effect of complete Freund's adjuvant in hypercholesterolemic *ldlr*^{-/-} and *apoe*^{-/-} mice (30-31). In a recent study, subcutaneous administration of alum adjuvant was shown to increase antigen uptake and activation of cellular immune responses in hypercholesterolemic mice (32). In some instances, a specific antibody response against the apoB-100 peptide and an immunomodulatory cytokine profile in aortas of mice immunized with OVA-CTB described herein is in line with such an adjuvant effect. This further underlines the importance of using optimal immunomodulatory components in vaccine preparations.

[0068] In several instances, atheroprotective vaccine is provided by targeting a peptide of the LDL protein constituent apolipoprotein B-100 to the nasal mucosa to induce a protective mucosal immune response.

[0069] Further details concerning the implementation of the fusion products methods herein described including systems for performance of the methods which can be in the form of kit of parts as well as related compositions including donors, acceptors, compounds and other reagents together with suitable carrier, agent or auxiliary agent of the compositions, and generally manufacturing and packaging of the kit, can be identified by the person skilled in the art upon reading of the present disclosure.

Examples

[0070] The fusion proteins and related compositions methods and systems herein described are further illustrated in the following examples, which are provided by way of illustration and are not intended to be limiting.

[0071] In particular, the following examples illustrate an exemplary immunization performed with a fusion protein comprising amino acids 3136-3155 of human apoB-100 (p210) fused with CTB identified also as p210-CTB. A person skilled in the art will appreciate the applicability of the features described in detail for P210-CTB for additional fusion protein comprising another antigenic peptide of apoB-100 and a carrier molecule according to the present disclosure.

[0072] More particular, in the following examples the recombinant protein, p210-CTB, was made from amino acids 3136-3155 of human apoB-100 (p210)¹⁵ fused with CTB. This sequence is identical to the corresponding murine sequence with the exception of a 2-residue insert at the C-terminal end in the mouse. As a control, amino acids 323-339 of ovalbumin were fused to CTB (OVA-CTB). 8-week-old female *apoe*^{-/-} mice received a nasal spray with 15 µg (in 15 µL) p210-CTB or OVA-CTB twice weekly. Lesions and immune parameters were analyzed 12 weeks later. In another set of experiments, *apoe*^{-/-} x CD4dnTGFβRII mice (23) were immunized using the same protocol. All experiments were approved by the Stockholm regional ethical board. Atherosclerotic lesions were analyzed in cryostat sections of the aortic root using a standardized protocol (24). Antibodies to p210 and to mouse LDL particles were analyzed by immunometric ELISA¹⁵.

[0073] Antigen-specific Treg activity was analyzed in the following way: *apoe*^{-/-} mice were immunized subcutaneously with apoB-100 to generate effector T cells. CD4⁺ T cells from these mice were exposed to antigen and activation recorded as DNA synthesis. CD4⁺ T cells from *apoe*^{-/-} mice immunized intranasally with p210-CTB were added to effector T cell preparations and Treg activity was recorded as inhibition of DNA synthesis. Intracellular staining was performed on CD4⁺ T cells to characterize cytokine production and T cell subtype.

[0074] Additional details concerning procedures used and results obtained are reported below.

Example 1: Generation of Peptide Library

[0075] Specific immunogenic epitopes by focusing on the single protein found in LDL, apolipoprotein B-100 (apo B) were characterized. A peptide library comprised of 302 peptides, 20 amino acid residues in length, covering the complete 4563 amino acid sequence of human apo B was produced. The peptides were produced with a 5 amino acid overlap to cover all sequences at break points. Peptides were numbered 1-302 starting at the N-terminal of apo B as indicated in **Table 1** below.

Table 1

Peptide	Sequence	Apolipoprotein B aa	SEQ ID NO
P1:	EEEML ENVSL VCPKD ATRFK	aa 1-20	1
P2:	ATRFK HLRKY TYNYE AESSS	aa 16-35	2
P3:	AESSS GVPGT ADSRS ATRIN	aa 31-50	3
P4:	ATRIN CKVEL EVPQL CSFIL	aa 46-65	4
P5:	CSFIL KTSQC TLKEV YGFNP	aa 61-80	5

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Peptide	Sequence	Apolipoprotein B aa	SEQ ID NO
P6:	YGFNP EGKAL LKKTK NSEEF	aa 76-95	6
P7:	NSEEF AAAMS RYELK LAIPE	aa 91-110	7
P8:	LAIPE GKQVF LYPEK DEPTY	aa 106-125	8
P9:	DEPTY ILNIK RGIIS ALLVP	aa 121-140	9
P10:	ALLVP PETEE AKQVL FLDTV	aa 136-155	10
P11:	FLDTV YGNCS THFTV KTRKG	aa 151-170	11
P12:	KTRKG NVATE ISTER DLGQC	aa 166-185	12
P13:	DLGQC DRFKP IRTGI SPLAL	aa 181-200	13
P14:	SPLAL IKGMT RPLST LISSS	aa 196-215	14
P15:	LISSS QSCQY TLDAK RKHVA	aa 211-230	15
P16:	RKHVA EAICK EQHLF LPFSY	aa 226-245	16
P17:	LPFSY NNKYG MVAQV TQTLK	aa 241-260	17
P18:	TQTLK LEDTP KINSR FFGEG	aa 256-275	18
P19:	FFGEG TKKMG LAFES TKSTS	aa 271-290	19
P20:	TKSTS PPKQA EAVLK TLQEL	aa 286-305	20
P21:	TLQEL KKLTI SEQNI QRANL	aa 301-320	21
P22:	QRANL FNKLTV TELRG LSDEA	aa 316-335	22
P23:	LSDEA VTSLL PQLIE VSSPI	aa 331-350	23
P24:	VSSPI TLQAL VQCGQ PQCST	aa 346-365	24
P25:	PQCST HILQW LKRVH ANPLL	aa 361-380	25
P26:	ANPLL IDVVT YLVAL IPEPS	aa 376-395	26
P27:	IPEPS AQQLR EIFNM ARDQR	aa 391-410	27
P28:	ARDQR SRATL YALSH AVNNY	aa 406-425	28
P29:	AVNNY HKTNP TGTQE LLDIA	aa 421-440	29
P30:	LLDIA NYLME QIQDD CTGDE	aa 436-455	30
P31:	CTGDE DYTIL ILRVI GNMGG	aa 451-470	31
P32:	GNMGG TMEQL TPELK SSILK	aa 466-485	32
P33:	SSILK CVQST KPSLM IQKAA	aa 481-500	33
P34:	IQKAA IQALR KMEPK DKDQE	aa 496-515	34
P35:	DKDQE VLLQT FLDDA SPGDK	aa 511-530	35
P36:	SPGDK RLAAY LMLMR SPSQA	aa 526-545	36
P37:	SPSQA DINKI VQILP WEQNE	aa 541-560	37
P38:	WEQNE QVKNF VASHI ANILN	aa 556-575	38
P39:	ANILN SEELD IQDLK KLVKE	aa 571-590	39
P40:	KLVKE ALKES QLPTV MDFRK	aa 586-605	40
P41:	MDFRK FSRNY QLYKS VSLPS	aa 601-620	41
P42:	VSLPS LDPAS AKIEG NLIFD	aa 616-635	42
P43:	NLIFD PNYYL PKESM LKTTL	aa 631-650	43

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(continued)

Peptide	Sequence	Apolipoprotein B aa	SEQ ID NO
P44:	LKTTL TAFGF ASADL IEIGL	aa 646-665	44
P45:	IEIGL EGKGF EPTLE ALFGK	aa 661-680	45
P46:	ALFGK QGFFP DSVNK ALYWV	aa 676-695	46
P47:	ALYWV NGQVP DGVSK VLVDH	aa 691-710	47
P48:	VLVDH FGYTK DDKHE QDMVN	aa 706-725	48
P49:	QDMVN GIMLS VEKLI KDLKS	aa 721-740	49
P50:	KDLKS KEVPE ARAYL RILGE	aa 736-755	50
P51:	RILGE ELGFA SLHDL QLLGK	aa 751-770	51
P52:	QLLGK LLLMG ARTLQ GIPQM	aa 766-785	52
P53:	GIPQM IGEVI RKGSK NDFFL	aa 781-800	53
P54:	NDFFL HYIFM ENAFE LPTGA	aa 796-815	54
P55:	LPTGA GLQLQ ISSSG VIAPG	aa 811-830	55
P56:	VIAPG AKAGV KLEVA NMQAE	aa 826-845	56
P57:	NMQAE LVAKP SVSVE FVTNM	aa 841-860	57
P58:	FVTNM GIIIP DFARS GVQMN	aa 856-875	58
P59:	GVQMN TNFFH ESGLE AHVAL	aa 871-890	59
P60:	AHVAL KAGKL KFIIP SPKRP	aa 886-905	60
P61:	SPKRP VKLLS GGNTL HLVST	aa 901-920	61
P62:	HLVST TKTEV IPPLI ENRQS	aa 916-935	62
P63:	ENRQS WSVCK QVFPQ LNYCT	aa 931-950	63
P64:	LNYCT SGAYS NASST DSASY	aa 946-965	64
P65:	DSASY YPLTG DTRLE LELRP	aa 961-980	65
P66:	LELRP TGEIE QYSVS ATYEL	aa 976-995	66
P67:	ATYEL QREDR ALVDT LKFVT	aa 991-1010	67
P68:	LKFVT QAEGA KQTEA TMTFK	aa 1006-1025	68
P69:	TMTFK YNRQS MTLSS EVQIP	aa 1021-1040	69
P70:	EVQIP DFDVD LGTIL RVNDE	aa 1036-1055	70
P71:	RVNDE STEGK TSYRL TLDIQ	aa 1051-1070	71
P72:	TLDIQ NKKIT EVALM GHLSC	aa 1066-1085	72
P73:	GHLSC DTKEE RKIKG VISIP	aa 1081-1100	73
P74:	VISIP RLQAE ARSEI LAHWS	aa 1096-1115	74
P75:	LAHWS PAKLL LQMDS SATAY	aa 1111-1130	75
P76:	SATAY GSTVS KRVAW HYDEE	aa 1126-1145	76
P77:	HYDEE KIEFE WNTGT NVDTK	aa 1141-1160	77
P78:	NVDTK KMTSN FPDVL SDYPK	aa 1156-1175	78
P79:	SDYPK SLHMY ANRLL DHRVP	aa 1171-1190	79
P80:	DHRVP ETDMT FRHVG SKLIV	aa 1186-1205	80
P81:	SKLIV AMSSW LQKAS GSLPY	aa 1201-1220	81

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(continued)

Peptide	Sequence	Apolipoprotein B aa	SEQ ID NO
P82:	GSLPY TQTLQ DHLNS LKEFN	aa 1216-1235	82
P83:	LKEFN LQNMG LPDFH IPENL	aa 1231-1250	83
P84:	IPENL FLKSD GRVKY TLNKN	aa 1246-1260	84
P85:	TLNKN SLKIE IPLPF G GKSS	aa 1261-1280	85
P86:	G GKSS RDLKM LETVR TPALH	aa 1276-1295	86
P87:	TPALH FKSVG FHLPS REFQV	aa 1291-1310	87
P88:	REFQV PTFTI PKLYQ LQVPL	aa 1306-1325	88
P89:	LQVPL LGVLD LSTNV YSNLY	aa 1321-1340	89
P90:	YSNLY NWSAS YSGGN TSTDH	aa 1336-1355	90
P91:	TSTDH FSLRA RYHMK ADSVV	aa 1351-1370	91
P92:	ADSVV DLLSY NVQGS GETTY	aa 1366-1385	92
P93:	GETTY DHKNT FTLSC DGSLR	aa 1381-1400	93
P94:	DGSLR HKFLD SNIKF SHVEK	aa 1396-1415	94
P95:	SHVEK LGNNP VSKGL LIFDA	aa 1411-1430	95
P96:	LIFDA SSSWG PQMSA SVHLD	aa 1426-1445	96
P97:	SVHLD SKKKQ HLFVK EVKID	aa 1441-1460	97
P98:	EVKID GQFRV SSFYA KGTYG	aa 1456-1475	98
P99:	KGTYG LSCQR DPNTG RLNGE	aa 1471-1490	99
P100:	RLNGE SNLRF NSSYL QGTNQ	aa 1486-1505	100
P101:	QGTNQ ITGRY EDGTL SLTST	aa 1501-1520	101
P102:	SLTST SDLQS GIIKN TASLK	aa 1516-1535	102
P103:	TASLK YENYE LTLKS DTNGK	aa 1531-1550	103
P104:	DTNGK YKNFA TSNKM DMTFS	aa 1546-1565	104
P105:	DMTFS KQNAL LRSEY QADYE	aa 1561-1580	105
P106:	QADYE SLRFF SLLSG SLNSH	aa 1576-1595	106
P107:	SLNSH GLELN ADILG TDKIN	aa 1591-1610	107
P108:	TDKIN SGAHK ATLRI GQDGI	aa 1606-1625	108
P109:	GQDGI STSAT TNLKC SLLVL	aa 1621-1640	109
P110:	SLLVL ENELN AELGL SGASM	aa 1636-1655	110
P111:	SGASM KLTTN GRFRE HNAKF	aa 1651-1670	111
P112:	HNAKF SLDGK AALTE LSLGS	aa 1666-1685	112
P113:	LSLGS AYQAM ILGVD SKNIF	aa 1681-1700	113
P114:	SKNIF NFKVS QEGLK LSNDM	aa 1696-1715	114
P115:	LSNDM MGSYA EMKFD HTNSL	aa 1711-1730	115
P116:	HTNSL NIAGL SLDFS SKLDN	aa 1726-1745	116
P117:	SKLDN IYSSD KFYKQ TVNLQ	aa 1741-1760	117
P118:	TVNLQ LQPYS LVTTT NSDLK	aa 1756-1775	118
P119:	NSDLK YNALD LTNNG KLRLE	aa 1771-1790	119

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Peptide	Sequence	Apolipoprotein B aa	SEQ ID NO
P120:	KLRLE PLKLH VAGNL KGAYQ	aa 1786-1805	120
P121:	KGAYQ NNEIK HIYAI SSAAL	aa 1801-1820	121
P122:	SSAAL SASYK ADTVA KVQGV	aa 1816-1835	122
P123:	KVQGV EFSHR LNTDI AGLAS	aa 1831-1850	123
P124:	AGLAS AIDMS TNYNS DSLHF	aa 1846-1865	124
P125:	DSLHF SNVFR SVMAP FTMTI	aa 1861-1880	125
P126:	FTMTI DAHTN GNGKL ALWGE	aa 1876-1895	126
P127:	ALWGE HTGQL YSKFL LKAEP	aa 1891-1910	127
P128:	LKAEP LAFTF SHDYK GSTSH	aa 1906-1925	128
P129:	GSTSH HLVSR KSISA ALEHK	aa 1921-1940	129
P130:	ALEHK VSALL TPAEQ TGTWK	aa 1936-1955	130
P131:	TGTWK LKTQF NNNEY SQDLD	aa 1951-1970	131
P132:	SQDLD AYNTK DKIGV ELTGR	aa 1966-1985	132
P133:	ELTGR TLADL TLLDS PIKVP	aa 1981-2000	133
P134:	PIKVP LLLSE PINII DALEM	aa 1996-2015	134
P135:	DALEM RDAVE KPQEF TIVAF	aa 2011-2030	135
P136:	TIVAF VKYDK NQDVH SINLP	aa 2026-2045	136
P137:	SINLP FFETL QEYFE RNRQT	aa 2041-2060	137
P138:	RNRQT IIVVV ENVQR NLKHI	aa 2056-2075	138
P139:	NLKHI NIDQF VRKYR AALGK	aa 2071-2090	139
P140:	AALGK LPQQA NDYLN SFNWE	aa 2086-2105	140
P141:	SFNWE RQVSH AKEKL TALTK	aa 2101-2120	141
P142:	TALTK KYRIT ENDIQ IALDD	aa 2116-2135	142
P143:	IALDD AKINF NEKLS QLQTY	aa 2131-2150	143
P144:	QLQTY MIQFD QYIKD SYDLH	aa 2146-2165	144
P145:	SYDLH DLKIA IANII DEIIE	aa 2161-2180	145
P146:	DEIIE KLKSL DEHYH IRVNL	aa 2176-2195	146
P147:	IRVNL VKTIH DLHLF IENID	aa 2191-2210	147
P148:	IENID FNKSG SSTAS WIQNV	aa 2206-2225	148
P149:	WIQNV DTKYQ IRIQI QEKLQ	aa 2221-2240	149
P150:	QEKLQ QLKRH IQNID IQHLA	aa 2236-2255	150
P151:	IQHLA GKLKQ HIEAI DVRVL	aa 2251-2270	151
P152:	DVRVL LDQLG TTISF ERIND	aa 2266-2285	152
P153:	ERIND VLEHV KHFVI NLIGD	aa 2281-2300	153
P154:	NLIGD FEVAE KINAF RAKVH	aa 2296-2315	154
P155:	RAKVH ELIER YEVDQ QIQVL	aa 2311-2330	155
P156:	QIQVL MDKLV ELTHQ YKLKE	aa 2326-2345	156
P157:	YKLKE TIQKL SNVLQ QVKIK	aa 2341-2360	157

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Peptide	Sequence	Apolipoprotein B aa	SEQ ID NO
P158:	QVKIK DYFEK LVGFI DDAVK	aa 2356-2375	158
P159:	DDAVK KLNEL SFKTF IEDVN	aa 2371-2390	159
P160:	IEDVN KFLDM LIKKL KSFDY	aa 2386-2405	160
P161:	KSFDY HQFVD ETNDK IREVT	aa 2401-2420	161
P162:	IREVT QRLNG EIQUAL ELPQK	aa 2416-2435	162
P163:	ELPQK AEALK LFLEE TKATV	aa 2431-2450	163
P164:	TKATV AVYLE SLQDT KITLI	aa 2446-2465	164
P165:	KITLI INWLQ EALSS ASLAH	aa 2461-2480	165
P166:	ASLAH MKAKF RETLE DTRDR	aa 2476-2495	166
P167:	DTRDR MYQMD IQQEL QRYLS	aa 2491-2510	167
P168:	QRYLS LVGQV YSTLV TYISD	aa 2506-2515	168
P169:	TYISD WWTLA AKNLT DFAEQ	aa 2521-2540	169
P170:	DFAEQ YSIQD WAKRM KALVE	aa 2536-2555	170
P171:	KALVE QGFTV PEIKT ILGTM	aa 2551-2570	171
P172:	ILGTM PAFEV SLQAL QKATF	aa 2566-2585	172
P173:	QKATF QTPDF IVPLT DLRIP	aa 2581-2600	173
P174:	DLRIP SVQIN FKDLK NIKIP	aa 2596-2615	174
P175:	NIKIP SRFST PEFTI LNTFH	aa 2611-2630	175
P176:	LNTFH IPSFT IDVE MKVKI	aa 2626-2645	176
P177:	MVKI IRTID QMQNS ELQWP	aa 2641-2660	177
P178:	ELQWP VPDYI LRDLE VEDIP	aa 2656-2675	178
P179:	VEDIP LARIT LPDFR LPEIA	aa 2671-2690	179
P180:	LPEIA IPEFI IPTLN LNDFQ	aa 2686-2705	180
P181:	LNDFQ VPDLE IPEFQ LPHIS	aa 2701-2720	181
P182:	LPDIS HTIEV PTFGK LYSIL	aa 2716-2735	182
P183:	LYSIL KIQSP LFTLD ANADI	aa 2731-2750	183
P184:	ANADI GNGTT SANEI GIAAS	aa 2746-2765	184
P185:	GIAAS ITAKG ESKLE VLNFD	aa 2761-2780	185
P186:	VLNFD FQANA QLSNP KINPL	aa 2776-2795	186
P187:	KINPL ALKES VKFSS KYLRT	aa 2791-2810	187
P188:	KYLRT EHGSE MLFFG NAIEG	aa 2806-2825	188
P189:	NAIEG KSNTV ASLHT EKNTL	aa 2821-2840	189
P190:	EKNTL ELSNG VIVKI NNQLT	aa 2836-2855	190
P191:	NNQLT LDSNT KYFHK LNIPK	aa 2851-2870	191
P192:	LNIPK LDFSS QADLR NEIKT	aa 2866-2885	192
P193:	NEIKT LLKAG HIAWT SSGKG	aa 2881-2900	193
P194:	SSGKG SWKWA CPRFS DEGTH	aa 2896-2915	194
P195:	DEGTH ESQIS FTIEG PLTSF	aa 2911-2930	195

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Peptide	Sequence	Apolipoprotein B aa	SEQ ID NO
P196:	PLTSF GLSNK INSKH LRVNQ	aa 2926-2945	196
P197:	LRVNQ NLVYE SGSLN FSKLE	aa 2941-2960	197
P198:	FSKLE IQSQV DSQHV GHSVL	aa 2956-2975	198
P199:	GHSVL TAKGM ALFGE GKAEF	aa 2971-2990	199
P200:	GKAEF TGRHD AHLNG KVIGT	aa 2986-3005	200
P201:	KVIGT LKNSL FFSAQ PFEIT	aa 3001-3020	201
P202:	PFEIT ASTNN EGNLK VRFPL	aa 3016-3035	202
P203:	VRFPL RLTGK IDFLN NYALF	aa 3031-3050	203
P204:	NYALF LSPSA QQASW QVSAR	aa 3046-3065	204
P205:	QVSAR FNQYK YNQNF SAGNN	aa 3061-3080	205
P206:	SAGNN ENIME AHVGI NGEAN	aa 3076-3095	206
P207:	NGEAN LDFLN IPLTI PEMRL	aa 3091-3110	207
P208:	PEMRL PYTII TTPPL KDFSL	aa 3106-3125	208
P209:	KDFSL WEKTG LKEFL KTTKQ	aa 3121-3140	209
P210:	KTTKQ SFDLS VKAQY KKNKH	aa 3136-3155	210
P211:	KKNKH RHSIT NPLAV LCEFI	aa 3151-3170	211
P212:	LCEFI SQSIK SFDRH FEKNR	aa 3166-3185	212
P213:	FEKNR NNALD FVTKS YNETK	aa 3181-3200	213
P214:	YNETK IKFDK YKAEK SHDEL	aa 3196-3215	214
P215:	SHDEL PRTFQ IPGYT VPVVN	aa 3211-3230	215
P216:	VPVVN VEVSP FTIEM SAFGY	aa 3226-3245	216
P217:	SAFGY VFPKA VSMPs FSILG	aa 3241-3260	217
P218:	FSILG SDVRV PSYTL ILPSL	aa 3256-3275	218
P219:	ILPSL ELPVL HVPRN LKLSL	aa 3271-3290	219
P220:	LKLSL PHFKE LCTIS HIFIP	aa 3286-3305	220
P221:	HIFIP AMGNI TYDFS FKSSV	aa 3301-3320	221
P222:	FKSSV ITLNT NAELF NQSDI	aa 3316-3335	222
P223:	NQSDI VAHLL SSSSS VIDAL	aa 3331-3350	223
P224:	VIDAL QYKLE GTTRL TRKRG	aa 3346-3365	224
P225:	TRKRG LKLAT ALSLS NKFVE	aa 3361-3380	225
P226:	NKFVE GSHNS TVSLT TKNME	aa 3376-3395	226
P227:	TKNME VSVAK TTKAE IPILR	aa 3391-3410	227
P228:	IPILR MNFKQ ELNGN TSKP	aa 3406-3425	228
P229:	TSKP TVSSS MEFKY DFNSS	aa 3421-3440	229
P230:	DFNSS MLYST AKGAV DHKLS	aa 3436-3455	230
P231:	DHKLS LESLT SYFSI ESSTK	aa 3451-3470	231
P232:	ESSTK GDVKG SVLSR EYSGT	aa 3466-3485	232
P233:	EYSGT IASEA NTYLN SKSTR	aa 3481-3500	233

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(continued)

Peptide	Sequence	Apolipoprotein B aa	SEQ ID NO
P234:	SKSTR SSVKL QGTSK IDDIW	aa 3496-3515	234
P235:	IDDIW NLEVK ENFAG EATLQ	aa 3511-3530	235
P236:	EATLQ RIYSL WEHST KNHLQ	aa 3526-3545	236
P237:	KNHLQ LEGLF FTNGE HTSKA	aa 3541-3560	237
P238:	HTSKA TLELS PWQMS ALVQV	aa 3556-3575	238
P239:	ALVQV HASQP SSFHD FPDLG	aa 3571-3590	239
P240:	FPDLG QEVAL NANTK NQKIR	aa 3586-3605	240
P241:	NQKIR WKNEV RIHSG SFQSQ	aa 3601-3620	241
P242:	SFQSQ VELSN DQEK A HLDIA	aa 3616-3635	242
P243:	HLDIA GSLEG HLRFL KNIIL	aa 3631-3650	243
P244:	KNIIL PVYDK SLWDF LKLDV	aa 3646-3665	244
P245:	LKLDV TTSIG RRQHL RVSTA	aa 3661-3680	245
P246:	RVSTA FVYTK NPNGY SFSIP	aa 3676-3695	246
P247:	SFSIP VKVLA DKFIT PGLKL	aa 3691-3710	247
P248:	PGLKL NDLNS VLVMP TFHVP	aa 3706-3725	248
P249:	TFHVP FTDLQ VPSCK LDFRE	aa 3721-3740	249
P250:	LDFRE IQIYK KLRTS SFALN	aa 3736-3755	250
P251:	SFALN LPTLP EVKFP EVDVL	aa 3751-3770	251
P252:	EVDVL TKYSQ PEDSL IPFFE	aa 3766-3785	252
P253:	IPFFE ITVPE SQLTV SQFTL	aa 3781-3800	253
P254:	SQFTL PKSVS DGIAA LDLNA	aa 3796-3815	254
P255:	LDLNA VANKI ADFEL PTIIV	aa 3811-3830	255
P256:	PTIIV PEQTI EIPSI KFSVP	aa 3826-3845	256
P257:	KFSVP AGIVI PSFQA LTARF	aa 3841-3860	257
P258:	LTARF EVDSP VYNAT WSASL	aa 3856-3875	258
P259:	WSASL KNKAD YVETV LDSTC	aa 3871-3890	259
P260:	LDSTC SSTVQ FLEYE LNVLG	aa 3886-3905	260
P261:	LNVLG THKIE DGTLA SKTKG	aa 3901-3920	261
P262:	SKTKG TLAHR DFSAE YEEDG	aa 3916-3935	262
P263:	YEEDG KFEG L QEWE G KAHLN	aa 3931-3950	263
P264:	KAHLN IKSPA FTDLH LRYQK	aa 3946-3965	264
P265:	LRYQK DKKGI STSAA SPAVG	aa 3961-3980	265
P266:	SPAVG TVGMD MDEDD DFSKW	aa 3976-3995	266
P267:	DFSKW NFYYS PQSSP DKKLT	aa 3991-4010	267
P268:	DKKLT IFKTE LRVRE SDEET	aa 4006-4025	268
P269:	SDEET QIKVN WEEEA ASGLL	aa 4021-4040	269
P270:	ASGLL TSLKD NVPKA TGVLY	aa 4036-4055	270
P271:	TGVLY DYV NK YHWEH TGLTL	aa 4051-4070	271

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(continued)

Peptide	Sequence	Apolipoprotein B aa	SEQ ID NO
P272:	TGLTL REVSS KLRRN LQNNA	aa 4066-4085	272
P273:	LQNNA EWVYQ GAIRQ IDDID	aa 4081-4100	273
P274:	IDDID VRFQK AASGT TGTYQ	aa 4096-4115	274
P275:	TGTYQ EWKDK AQNLY QELLT	aa 4111-4130	275
P276:	QELLT QEGQA SFQGL KDNVF	aa 4126-4145	276
P277:	KDNVF DGLVR VTQKF HMKVK	aa 4141-4160	277
P278:	HMKVK HLIDS LIDFL NFPRF	aa 4156-4175	278
P279:	NFPRF QFPGK PGIYT REELC	aa 4171-4190	279
P280:	REELC TMFIR EVGTV LSQVY	aa 4186-4205	280
P281:	LSQVY SKVHN GSEIL FSYFQ	aa 4201-4220	281
P282:	FSYFQ DLVIT LPFEL RKHKL	aa 4216-4235	282
P283:	RKHKL IDVIS MYREL LKDLS	aa 4231-4250	283
P284:	LKDLS KEAQE VFKAQ QSLKT	aa 4246-4265	284
P285:	QSLKT TEVLR NLQDL LQFIF	aa 4261-4280	285
P286:	LQFIF QLIED NIKQL KEMKF	aa 4276-4295	286
P287:	KEMKF TYLIN YIQDE INTIF	aa 4291-4310	287
P288:	INTIF NDYIP YVFKL LKENL	aa 4306-4325	288
P289:	LKENL CLNLH KFNEF IQNEL	aa 4321-4340	289
P290:	IQNEL QEASQ ELQQI HQYIM	aa 4336-4355	290
P291:	HQYIM ALREE YFDPS IVGWT	aa 4351-4370	291
P292:	IVGWT VKYYE LEEKI VSLIK	aa 4366-4385	292
P293:	VSLIK NLLVA LKDFH SEYIV	aa 4381-4400	293
P294:	SEYIV SASNF TSQLS SQVEQ	aa 4396-4415	294
P295:	SQVEQ FLHRN IQEYL SILTD	aa 4411-4430	295
P296:	SILTD PDGKG KEKIA ELSAT	aa 4426-4445	296
P297:	ELSAT AQEII KSQAI ATKKI	aa 4441-4460	297
P298:	TKKII SDYHQ QFRYK LQDFS	aa 4457-4476	298
P299:	LQDFS DQLSD YYEKF IAESK	aa 4472-4491	299
P300:	IAESK RLIDL SIQNY HTFLI	aa 4487-4506	300
P301:	HTFLI YITEL LKKLQ STTVM	aa 4502-4521	301
P302:	STTVM NPYMK LAPGE LTIIL	aa 4517-4536	302

Example 2: ApoB-100-Peptides selection

[0076] Plasma samples were obtained from 10 patients with clinically evident atherosclerotic heart disease. In addition, 50 plasma samples were obtained from 25 men and 25 women with no evidence of atherosclerotic heart disease. Samples of the 20 amino acid long peptides were adsorbed to microtiter plates to perform enzyme-linked immunosorbent assay (ELISA) analyses of the plasma samples. Peptides were used in their native state or after oxidation by exposure to copper or after modification by malondialdehyde (MDA). Plasma samples from patients and normal subjects contained antibodies to a large number of different peptides. Antibodies against both native and modified peptides were identified. A total of 38 peptide sequences were identified as potential targets for immune reactions that may be of importance for

the development of atherosclerosis.

[0077] The peptide sequences against which the highest antibody levels were detected could be divided in six groups with certain common characteristics as indicated in **Table 2** below.

Table 2

Peptide	Sequence	Apo B-100 Amino Acid	SEQ ID NO
A. High levels of IgG antibodies to MDA modified peptides (n=3)			
P11	FLDTV-YGNCS-THFTV-KTRKG	151-170	11
P25	PQCST-HILQW-LKRVH-ANPLL	361-380	25
P74	VISIP-RLQAE-ARSEI-LAHWS	1096-1115	74
B. High levels of IgM antibodies, no difference between native and MDA-modified peptides (n=9)			
P40	KLVKE-ALKES-QLPTV-MDFRK	586-605	40
P94	DGSLR-HKFLD-SNIKF-SHVEK	1396-1415	94
P99	KGTYG-LSCQR-DPNTG-RLNGE	1471-1490	99
P100	RLNGE-SNLRP-NSSYL-QGTNQ	1486-1505	100
P102	SLTST-SDLQS-GIIKN-TASLK	1516-1535	102
P103	TASLK-YENYE-LTLKS-DTNGK	1531-1550	103
P105	DMTFS-KQANL-LRSEY-QADYE	1561-1580	105
P177	MKVKI-IRTID-QMQNS-ELQWP	2641-2660	177
C. High levels of IgG antibodies, no difference between native and MDA-modified peptides (n=2)			
P143	IALDD-AKINF-NEKLS-QLQTY	2131-2150	143
P210	KTTKQ-SFDLS-VKAQY-KKNKH	3136-3155	210
D. High levels of IgG antibodies to MDA-modified peptides and at least twice as much antibodies in the plasma of normal subjects as compared to the plasma of individuals with atherosclerosis (n=5)			
P1	EEEML-ENVSL-VCPKD-ATRFK	1-20	1
P129	GSTSH-HLVSR-KSISA-ALEHK	1921-1940	129
P148	IENID-FNKSG-SSTAS-WIQNV	2206-2225	148
P162	IREVT-QRLNG-EIQAL-ELPQK	2416-2435	162
P252	EVDVL-TKYSQ-PEDSL-IPFFE	3766-3785	252
E. High levels of IgM antibodies to MDA-modified peptides and at least twice as much antibodies in the plasma of normal subjects as compared to the plasma of individuals with atherosclerosis (n=11)			
P30	LLDIA-NYLME-QIQDD-CTGDE	436-455	30
P31	CTGDE-DYTYK-IKRVG-GNMGQ	451-470	31
P32	GNMGQ-TMEQL-TPELK-SSILK	466-485	32
P33	SSILK-CVQST-KPSLM-IQKAA	481-500	33
P34	IQKAA-IQALR-KMEPK-DKDQE	496-515	34
P100	RLNGE-SNLRP-NSSYL-QGTNQ	1486-1505	100
P107	SLNSH-GLELN-ADILG-TDKIN	1591-1610	107
P149	WIQNV-DTKYQ-IRIQI-QEKLQ	2221-2240	149
P169	TYISD-WWTLA-AKNLT-DFAEQ	2521-2540	169
P236	EATLQ-RIYSL-WEHST-KNHLQ	3526-3545	236
P301	HTFLI-YITEL-LKKLQ-STTVM	4501-4520	301

(continued)

F. High levels of IgG antibodies, but no difference between intact and MDA-modified peptides but at least twice as much antibodies in the plasma of individuals with atherosclerosis as compared to the plasma of normal subjects (n=7)

P10	ALLVP-PETEE-AKQVL-FLDTV	131-150	10
P45	IEIGL-EGKGF-EPTLE-ALFGK	661-680	45
P111	SGASM-KLTTN-GRFRE-HNAKF	1651-1670	111
P154	NLIGD-FEVAE-KINAF-RAKVH	2296-2315	154
P199	GHSVL-TAKGM-ALFGE-GKAEF	2971-2990	199
P222	FKSSV-ITLNT-NAELF-NQSDI	3316-3335	222
P240	FPDLG-QEVAL-NANTK-NQKIR	3586-3605	240

G. No level of IgG or IgM antibodies (n=1)

P2	ATRFK-HLRKY-TYNYE-AESSS	16-35	2
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Example 3: ApoB-100-Peptides selection

[0078] Inhibition of atherosclerosis in apo E $-/-$ mice by immunization with fifteen different test articles based on fifteen different peptide fragments of apo B was investigated.

Table 3A

Peptide	Sequence	Apo B-100 Amino Acid	SEQ ID NO
P2	ATRFK-HLRKY-TYNYE-AESSS	16-35	2
P11	FLDTV-YGNCS-THFTV-KTRKG	151-170	11
P25	PQCST-HILQW-LKRVH-ANPLL	361-380	25
P32	GNMGQ-TMEQL-TPELK-SSILK	466-485	32
P45	IEIGL-EGKGF-EPTLE-ALFGK	661-680	45
P74	VISIP-RLQAE-ARSEI-LAHWS	1096-1115	74
P102	SLTST-SDLQS-GIIKN-TASLK	1516-1535	102
P129	GSTSH-HLVSR-KSISA-ALEHK	1921-1940	129
P143	IALDD-AKINF-NEKLS-QLQTY	2131-2150	143
P148	IENID-FNKSG-SSTAS-WIQNV	2206-2225	148
P154	NLIGD-FEVAE-KINAF-RAKVH	2296-2315	154
P162	IREVT-QRLNG-EIQAL-ELPQK	2416-2435	162
P210	KTTKQ-SFDLS-VKAQY-KKNKH	3136-3155	210
P219	ILPSL-ELPVL-HVPRN-LKLSL	3271-3290	219
P240	FPDLG-QEVAL-NANTK-NQKIR	3586-3605	240

[0079] In these experiments, apo E $-/-$ mice received primary subcutaneous immunization at 6-7 weeks of age, followed by two boosters administered 3 and 5 weeks later. The mice were fed a high cholesterol diet from 1 week after the second injection (10 weeks of age) and continued until sacrifice at 25 weeks of age. The effect of immunization on atherosclerosis formation was assessed by measuring plaque size (percent area stained with Oil Red O) in an en face preparation of the aorta.

[0080] Based on the results from these and other experiments, four peptides were identified as particularly effective in reducing the progression of atherosclerosis.

Table 3B

Peptide	Reduction in atherosclerosis progression
P2	(-42%, p<0.05)
P45	(-53%, p<0.05)
P102	(-52%, p<0.05)
P210	(-49%, p=0.06)

Example 4: Cholera toxin B subunit gene fusions for immunization

[0081] The gene fusions used in the present disclosure were constructed using a CTB expression vector essentially as described previously (Sadeghi H, Bregenholt S, Wegmann D, Petersen JS, Holmgren J, and Lebens M. Genetic fusion of human insulin B-chain to the B-subunit of cholera toxin enhances in vitro antigen presentation and induction of bystander suppression in vivo. Immunology. 2002; 106:237-245). Synthetic oligonucleotides from Innovagen (Lund, Sweden) were used to make double stranded DNA fragments encoding the peptide sequence of interest that could be inserted into the vector such that the added peptide formed a carboxyl extension of mature CTB. The unmodified peptide p210 corresponding to amino acids 3136-3155 of human apoB-100 (KTTKQSFDSLVSVAQYKKKNKH - SEQ ID NO:210) was encoded by the DNA sequence:

5'CAAAACGACCAAGCAAAGCTTTGATCTGAGCGTGAAAGCGCAGTATAAGAAA
AACAAACACTA^{3'} (SEQ ID NO: 303)

^{3'}CATGGTTTTGCTGGTTCGTTTCGAAACTAGACTCGCACTTTTCGCGTCATATTCTT
TTTGTGTGATTCTGA^{5'} (SEQ ID NO: 304)

KTTKQSFDSLVSVAQYKKKNKH *** (SEQ ID NO: 210)

[0082] This sequence is 90.9% identical to amino acids 3157-3185 of the murine apoB100 sequence, the exception being the insertion of a Serine (S) and an Asp (D) residue between N and KH in the C-terminal portion of the peptide:

Human KTTKQSFDSLVSVAQYKKN-KH (SEQ ID NO:210)
Murine KTTKQSFDSLVSVAQYKKNSDKH (SEQ ID NO:305)

[0083] Oligonucleotides were synthesized that encoded the p210 peptide corresponding to amino acids 3136-3155 of human apoB-100. The coding regions are flanked by sticky ends compatible with restriction enzymes KpnI and HindIII. Insertion into the expression vector leads to an in-frame extension to the carboxyl terminus of mature CTB.

[0084] The synthetic sequence was optimized for expression in E. coli. The single strands were annealed and ligated into the pML-CTB vector digested with KpnI and HindIII. Ligated DNA was used to transform E. coli B strain BL21. Transformants were selected initially on the basis of ampicillin resistance and plasmids were then screened using restriction analysis. Finally the sequence of the insert in selected clones was confirmed by DNA sequencing. Protein expression was induced by addition of IPTG to the growth medium. This resulted in the production of insoluble inclusion bodies containing the recombinant protein. The cells were disrupted by sonication following treatment with lysozyme and DNase. The inclusion bodies could be separated from the soluble cell protein and other insoluble cell debris by low speed centrifugation. LPS was removed by washing the inclusion bodies in three times in 0.1% triton X114 in PBS at 4° C and subsequent extensive washing in PBS in order to remove the detergent. The inclusion bodies were dissolved in 6.5 M urea and reassembled into the biologically active pentameric form by removal of the urea by dialysis against 50 mM sodium carbonate buffer pH 9.0. The assembly and purity of the protein was assessed by SDS-PAGE. Receptor binding activity was confirmed by GM1 ELISA (Svennerholm AM, Holmgren J. Identification of Escherichia coli heat-labile enterotoxin by means of a ganglioside immunosorbent assay (GM1-ELISA) procedure. Curr Microbiol. 1978; 1:19-23). The protein was further partially purified by FPLC gel filtration using a sephadex 200 16/60 column. The OVA-CTB protein used as a control; also a fusion to the carboxyl terminus of mature CTB, was constructed and purified as previously described (George-Chandy A, Eriksson K, Lebens M, Nordström I, Schön E, Holmgren J. Cholera toxin B

subunit as a carrier molecule promotes antigen presentation and increases CD40 and CD86 expression on antigen presenting cells. Infect Immun. 2001; 69:5716-5725).

Example 5: Mice and immunization protocols

[0085] Female *apoe*^{-/-} mice were obtained from Taconic Europe (Ry, Denmark) and *Apoe*^{-/-} CD4dnTGFβRIItg mice were previously generated in our laboratory (ref. 19). Starting at 8 weeks of age, mice were immunized intranasally twice per week for 12 weeks with either p210-CTB or OVA-CTB, both at 15 μg/dose (15 μL volume), or left untreated (PBS). Mice were fed a normal laboratory diet and sacrificed by CO₂ asphyxiation. All experiments were approved by the regional board for animal ethics.

Example 6: Tissue processing and immunohistochemistry

[0086] Lesion area per cross-section and fractional area of the lesion in the aortic root were quantified and the results expressed as the mean of 5 sections per mouse (ref. 22). In brief, fractional lesion area is calculated for each section as $F\% = (100 \times L/A)$ where L is lesion area (μm²) and A is area inside external elastic lamina (μm²). F% is averaged over all levels analyzed (200-600 μm² above aortic cusps) and the mean calculated for each treatment group. This method eliminates artifacts caused by oblique sections.

[0087] Primary antibodies (CD4, CD68, VCAM-1, I-A^b; all rat anti-mouse from BD Biosciences (Franklin Lakes, NJ, U.S.A.) and FoxP3 by eBioscience (San Diego, CA, U.S.A.) titrated to optimum performance on spleen sections were applied to acetone-fixed cryosections from the aortic root, followed by detection with the ABC alkaline phosphatase kit from Vector Laboratories (Burlingame, CA, USA). A thresholding technique was implemented using computerized ImagePro analysis (Media Cybernetics, Bethesda, MD, U.S.A.) of immunostained sections. For RNA isolation the thoracic aortic arch distal of the aortic root was dissected and snap-frozen.

Example 7: Flow cytometry and Intracellular Cytokine Staining

[0088] Flow cytometry was performed on a CyAn™ (Dako, Glostrup, Denmark) after staining with the appropriate antibodies; data were analyzed using Summit v4.3 software (Dako). Primary labeled antibodies used were from BD Biosciences (anti-CD4) or from eBioscience (anti-FoxP3). To characterize the cytokine expression profiles of CD4⁺ T cells from lung and spleen of nasally vaccinated mice, cell suspensions were prepared as described before and evaluated by intracellular cytokine staining and FACS analysis. Briefly, lung mononuclear cells were isolated by collagenase Type I digestion (324U/ml; Sigma) for 1h on a shaker and splenocytes were prepared by mechanical disruption followed by incubation in erythrocyte lysis buffer (Qiagen, USA) and extensively washed. CD4⁺ T cells were purified using MACS magnetic cell separation as described above. 2 x 10⁵ spleen or lung cells previously stimulated with 10ug/ml of human apoB-100 for 24 hours, were restimulated for 4h at 37 °C in 7,5% CO₂ with PMA (phorbol 12-myristate 13-acetate; 50 ng/ml), ionomycin (1 ug/ml; Sigma) and GolgiPlug (lul per 1 ml; BD Bioscience).

[0089] Alternatively, 2 x 10⁵ CD4⁺ T cells previously stimulated with plate bound anti-CD3 (5 ug/ml) and anti-CD28 (2 ug/ml) for 3 days in culture together with recombinant mouse IL-2 (10 ng/ml; Peprotech) and IL-4 (1 ng/ml; Peprotech) followed by a 3 day incubation with only IL-2 and IL-4, were restimulated with plate bound anti-CD3 (5 ug/ml) and anti-CD28 (2 ug/ml) for 5h in the presence of GolgiPlug. All cells were incubated with FcγR block (BD Bioscience) followed by surface (anti-CD4) and intracellular staining of IFNγ, IL-4, IL-17 or IL-10 (BD Bioscience) and FoxP3 (eBioscience) according to the manufacturer's instructions. Cells were analyzed on a CyAn™ flow cytometer (Dako).

Example 8: Functional immunoassays

[0090] A first group of *apoe*^{-/-} mice were immunized subcutaneously with HPLC-purified human apoB-100 in complete Freund's adjuvant (CFA) from Pierce (Rockford, IL, U.S.A.) and boosted 4 weeks later with apoB-100 in incomplete Freund's adjuvant (IFA) from Pierce to generate spleen T cells sensitized to human apoB-100, which were harvested one week later. A second group of mice received the nasal vaccine over 2 weeks (4 doses total/mouse) and CD4⁺ T cells were harvested from the spleen 3 days after the last nasal administration of the vaccine. Spleen CD4⁺ T cells (> 95% purity) were isolated by negative selection over a magnetic column using MACS microbeads (CD4+ negative selection kit, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's protocol. Splenocytes from apoB100-vaccinated *apoe*^{-/-} mice were cocultured at varying dilution ratios with purified CD4⁺ T cells from spleens of mice that had received nasal p210-CTB, OVA-CTB or PBS. To exclude contaminating apoB-100 in cell culture media, FCS-free IMDM from Gibco (Invitrogen, Carlsbad, CA, U.S.A.) was supplemented with ITS™ from BD Biosciences. Cells were incubated for 72 hours in the absence or presence of purified human apoB-100 (20 μg/mL) with incorporation of ³H-thymidine during the last 18 hours. Data are presented as stimulation index (ratio of apoB-100-challenged to

unchallenged coculture assay). In a second approach splenocytes and purified CD4⁺ T cells were separated in transwell plates from Corning (Corning, NY, U.S.A.) to analyze whether cell-cell contact inhibition abrogated the suppressive effect of tolerized CD4⁺ T cells.

Example 9: Antibody assays

[0091] ELISA methods were used to quantitate serum Ig isotypes specific for the apoB-100 peptide as well as total IgG and IgM as previously described (ref. 19). Sera from immunized mice were tested for antibodies to mouse LDL by incubation (1/50, 1/150 and 1/450 dilutions) in plates coated with mouse LDL (10 µg/ml) and using alkaline phosphatase-conjugated anti-mouse-IgG as detector antibody. Sera from C57BL/6 mice immunized with OVA-CTB were assayed for reactivity to mouse or human LDL, or to apoB100, at dilutions of 1/25, 1/250 and 1/2500.

Example 10: Real-Time Polymerase Chain Reaction

[0092] RNA was isolated from the aortic arch using the RNeasy kit from Qiagen (Hilden, Germany). Total RNA was analyzed by BioAnalyzer from Agilent Technologies (Waldbronn, Germany). Reverse transcription was performed with Superscript-II and random hexamers (both from Invitrogen) and cDNA amplified by real-time PCR using primers and probes for FoxP3, IL-10, TGF-β, IFN-γ and hypoxanthine guanine ribonucleosyltransferase (HPRT) in an ABI 7700 Sequence Detector from Applied Biosystems. All primers and probes were obtained as "assays on demand" from Applied Biosystems (Foster City, CA, U.S.A.) Data were analyzed on the basis of the relative expression method with the formula $2^{-\Delta\Delta C_T}$, where $\Delta\Delta C_T = \Delta C_T (\text{sample}) - \Delta C_T (\text{calibrator})$ (average C_T values of all samples within each group), and ΔC_T is the C_T of the housekeeping gene (HPRT) subtracted from the C_T of the target gene.

Example 11: Serum analyses

[0093] Total serum triglycerides were determined with an enzymatic assay from Roche Diagnostics (Mannheim, Germany) using a TECAN InfiniTE M200 plate reader (TECAN Nordic, Täby Sweden). Total serum cholesterol and lipoprotein profiles were determined by FPLC separation of 2 µL serum of all individuals using a micro-FPLC column from GE Healthcare coupled to a system for online separation and subsequent detection of cholesterol as described, using human serum as reference (Parini P et al., Lipoprotein profiles in plasma and interstitial fluid analyzed with an automated gel-filtration system. Eur J Clin Invest 2006;36:98-104). IL-10 ELISA from Mabtech (Nacka Strand, Sweden) and TGF-β ELISA from R&D Systems (Minneapolis, MN, U.S.A.) was used to measure cytokine levels in supernatants.

Example 12: Statistical analysis

[0094] Values are expressed as mean ± standard error of the mean (SEM) unless otherwise indicated. Non-parametric Kruskal-Wallis test was used for multiple comparisons, Mann-Whitney U test was used for pairwise comparisons. A p-value of < 0.05 was considered significant.

Example 13: Nasal administration of p210-CTB inhibits atherosclerosis

[0095] Nasal immunization with p210-CTB caused a significant 35% reduction in atherosclerotic lesion size ($p = 0.015$; $p = 0.039$) and fractional lesion area ($p = 0.012$; $p = 0.007$) in the aortic root as compared with OVA-CTB or untreated controls, respectively (**Figure 1A,B** and **Figure 6**). Atherosclerosis was not attenuated by administration of OVA-CTB compared with untreated controls indicating an apoB-100 peptide-specific effect (**Figure 1**). The composition of the lesions was not significantly altered by p210-CTB immunization, as indicated by quantitative immunohistochemical analysis of markers for CD4⁺ T cells, macrophages (CD68), or the inducible surface proteins I-A^b (major histocompatibility complex class II protein) and the vascular cell adhesion molecule-1 (**Table 4**).

Table 4: Weight, cholesterol and triglyceride levels in plasma

	group	weight (g)	cholesterol (mg/dL)	triglycerides (mg/dL)
<i>apoe</i> ^{-/-}	p210-CTB	21 ± 1.3	281 ± 102	46 ± 9.1
	OVA-CTB	21.5 ± 0.6	257 ± 51	36 ± 3.9
	control	20.5 ± 1.0	313 ± 59	52 ± 7.9
<i>apoe</i> ^{-/-} × <i>CD4dn</i>	p210-CTB	19.1 ± 2.9	254 ± 84	40 ± 8.3
<i>TbRIItg</i>	OVA-CTB	20.8 ± 1.2	250 ± 94	40 ± 6.2

(continued)

group	weight (g)	cholesterol (mg/dL)	triglycerides (mg/dL)
p value	n.s	n.s.	n.s.

Mean values and standard deviations are shown. Non-parametric group comparisons were performed using the Kruskal-Wallis test.

Example 14: Nasal administration of p210-CTB does not affect plasma lipids

[0096] Immunization did not significantly affect body weight, serum cholesterol or triglycerides (Table 5). Plasma lipoprotein profiles were similar in mice immunized with p210-CTB or OVA-CTB, respectively (Figure 7).

Table 5: Cellular composition and inflammatory markers in aortas of 20 weeks old *apoe*^{-/-} mice

	P210-CTB (A)	OVA-CTB (B)	control	p value
CD4 (cells/mm ²)	176 ± 31	151 ± 35	124 ± 21	n.s.
I-A ^b (cells/mm ²)	87 ± 25	62 ± 17	112 ± 34	n.s.
CD68 (%lesion)	15.9 ± 3.6	22.6 ± 7.4	12.6 ± 2.3	n.s.
VCAM-1 (%lesion)	12.7 ± 4.6	22.3 ± 11.3	10.9 ± 1.0	n.s.
FoxP3 (cells/mm ²)	4.1 ± 0.8	4.2 ± 1.0	6.5 ± 2.6	n.s.

[0097] Positive cells are calculated per mm² lesion area except for Fox3 cells, which are presented as cells per total vessel surface area as they were also found in the adventitia. Statistics were performed using the non-parametric Kruskal-Wallis test.

Example 15: CTB fusion protein immunization increases aortic FoxP3 and IL-10 mRNA levels

[0098] Real-time reverse transcription-PCR analysis of the thoracic aorta of *apoe*^{-/-} mice showed significant increases in FoxP3 and IL-10 mRNA levels in both CTB vaccine groups (p210-CTB and OVA-CTB) (Figure 1C). No statistically relevant differences in FoxP3 or IL-10 mRNA were detected when comparing mice that had received p210-CTB or OVA-CTB, respectively. Furthermore, FoxP3⁺ cell numbers did not differ between groups; a representative example of FoxP3⁺ cells in aortic lesions is shown in Figure 8. IL-10 was elevated to the same extent in p210-CTB and OVA-CTB groups, pointing to a possible adjuvant effect of CTB. Trends towards increased TGF-β and decreased IFN-γ mRNA in vaccinated mice were not significant.

Example 16: Nasal vaccination induces mucosal and systemic humoral and cellular immune responses

[0099] P210-CTB immunization induced significantly elevated titers of IgG antibodies to the p210 peptide of apoB-100 (Figure 2A). Modestly increased IgG anti-p210 was observed in OVA-CTB immunized *apoe*^{-/-} mice. The IgG1/IgG2a ratio of anti-p210 antibodies did not change, implying that there was no Th1/Th2 shift in T helper activity to B cell activation (Figure 9 and Figure 10). Total IgG levels were not influenced by either treatment (Figure 11). p210-specific IgM titers were significantly elevated both in p210-CTB and OVA-CTB treated groups; however, no difference was detected between p210-CTB and OVA-CTB treated animals (Figure 2B). Total IgM was not influenced by either treatment (Figure 12). Sera of immunized mice were tested for antibodies to mouse LDL particles, however, ELISA did not show any such titers (data not shown). Therefore, antibodies induced to human p210 did not recognize intact, endogenous LDL particles in the immunized mice. *Apoe*^{-/-} mice immunized with OVA-CTB showed modestly increased titers to p210 (Figure 2). However, parenteral immunization with OVA did not lead to induction of significant IgG antibody responses to mouse LDL (data not shown), thus ruling out serological crossreactivity between OVA and LDL protein.

[0100] Analysis of the cellular immune response in the lung, the major organ targeted after nasal vaccination, showed a significant decrease in CD4⁺ T cells expressing interferon-γ (characteristic of Th1 cells) and IL-17 (characteristic of Th17 cells), respectively, in mice treated with p210-CTB (Figure 3 A,B). In contrast, no such change was recorded for IL-4⁺ CD4⁺ T cells or for FoxP3⁺ CD4⁺ T cells (Figure 3 C,D). This indicates a shift of the T helper cell balance in the respiratory mucosa, away from the proinflammatory Th1 and Th17 subtypes after nasal immunization with p210-CTB.

[0101] Systemic cellular immune responses were monitored in spleen cell preparations. Nasal immunization with p210-CTB significantly increased the proportion of spleen CD4⁺ T cells expressing the anti-inflammatory cytokine IL-10

(Figure 4 A and B). Unlike the situation in the lung, no significant differences were detected in the distribution of the remaining CD4⁺ T cell subsets in the spleen, as characterized by intracellular staining for interferon- γ , IL-17, IL-4 and FoxP3 (Figure 13).

Example 17: P210-CTB treatment induces ApoB100-specific Treg activity

[0102] To assess whether functional Treg were induced by immunization, we exposed spleen CD4⁺ T cells from *apoe*^{-/-} mice immunized subcutaneously with human apoB-100 (effector T cells), to CD4⁺ T cells from mice immunized nasally with either p210-CTB, OVA-CTB, or no antigen (Figure 4C). A marked dose-dependent inhibition of effector T cell proliferation was observed in the presence of CD4⁺ T cells from p210-CTB immunized mice. No such inhibition was observed when T cells from OVA-CTB or non-immunized mice were added. The inhibitory effect of T cells from p210-CTB immunized mice was abolished when these cells were separated from effector T cells by a membrane, indicating that suppression required cell-cell contact (Figure 14). Levels of IL-10 and TGF- β in culture supernatants did not differ between groups (Figure 15).

Example 18: The atheroprotective effect of nasal p210-CTB vaccination is independent of TGF- β signaling in T cells

[0103] To determine whether the atheroprotective effect of nasal vaccination with p210-CTB depended on TGF- β signaling in T cells, we immunized *apoe*^{-/-} mice lacking functional TGF- β receptors on T cells (CD4dnTGF β RII x *apoe*^{-/-} mice). Nasal immunization with p210-CTB significantly reduced atherosclerotic lesion size by 30% in CD4dnTGF β RII x *apoe*^{-/-} mice, as compared with littermates immunized with OVA-CTB (Figure 5A and Figure 16). This indicates that TGF β R signaling in T cells is not required for the atheroprotective effect of nasal p210-CTB vaccination. It also argues against a decisive role for FoxP3⁺ Treg, as these cells are thought to require TGF β for their function. IgG but not IgM antibodies directed against the apoB100-peptide were significantly elevated in all groups of mice immunized with p210-CTB, irrespective of whether signaling via TGF- β was blocked during immunization (Figure 5B,C). Analysis of mRNA expression in aortas showed no differences in mRNA for, IL-10, TGF- β or interferon- γ between p210-CTB- and OVA-CTB-vaccinated mice (Figure 17). Surprisingly, FoxP3 mRNA was not reduced in CD4dnTGF β RII x *apoe*^{-/-} mice, possibly reflecting the presence of immature Treg (Figure 17). Furthermore, quantitative immunohistochemistry showed no differences in cellular composition of lesions between treatment groups (Table 6).

Table 6: Cellular composition and inflammatory markers in aortas of 20 weeks old *apoe*^{-/-}CD4dnTGF β RII^{tg} mice

	p210-CTB (C)	OVA-CTB (D)	p value
CD4 (cells/mm ²)	120 $\pm$ 43	173 $\pm$ 55	n.s.
I-A ^b (cells/mm ²)	94 $\pm$ 16	238 $\pm$ 102	n.s.
CD68 (%lesion)	9.9 $\pm$ 2.8	14.5 $\pm$ 2.6	n.s.
VCAM-1 (%lesion)	7.6 $\pm$ 2.6	19.3 $\pm$ 2.2	n.s.
FoxP3 (cells/mm ²)	19.7 $\pm$ 5.2	25.6 $\pm$ 3.4	n.s.

[0104] Positive cells are calculated per mm² lesion area except for FoxP3 cells, which are presented as cells per total vessel surface area as they were also found in the adventitia. Statistics were performed using the non-parametric Kruskal Wallis test.

[0105] In the above exemplary procedures, a peptide comprising amino acids 3136-3155 of apolipoprotein B-100 (p210) was fused to the B subunit of cholera toxin (CTB), which binds to a ganglioside on mucosal epithelia. The effect of nasal administration of the p210-CTB fusion protein on atherogenesis was compared with that of an ovalbumin peptide fused to CTB and with untreated controls. Immunization with p210-CTB for 12 weeks caused a 35% reduction in aortic lesion size of *apoe*^{-/-} mice. This effect was accompanied by induction regulatory T cells that markedly suppressed effector T cells rechallenged with apoB-100 and increased numbers of IL-10⁺ CD4⁺ T cells. Furthermore, a peptide-specific antibody response was observed. Atheroprotection was also documented in *apoe*^{-/-} mice lacking functional transforming growth factor-beta receptors on T cells.

[0106] The above results confirm and extend previous reports on atheroprotective effects of immunization with LDL or its components^{5-8, 12, 15, 16}. The use of complete LDL particles as immunogens is not attractive for clinical vaccination strategies since these particles may contain multiple pro-inflammatory and even potentially toxic molecules such as modified lipids and endotoxins. Recent studies have identified a set of apoB-100-derived peptides with significant atheroprotective effects^{15, 16}, enabling development of a structurally defined vaccine candidate. Among them, specific native peptides were immunogenic in humans and mice and correlated with the extent of atherosclerotic disease (33-34). By

combining a limited number of peptides in the vaccine, overcoming MHC restriction is expected. Combining peptide sequences with immunomodulatory components (adjuvants) such as CTB is an attractive approach to selectively induce protective immunity while avoiding side effects caused by non-peptide components in LDL particles. Unlike LDL, the vaccine formulation can be manufactured in a reproducible way and under Good Manufacturing Practice (GMP) conditions. The possibility to induce atheroprotective immunity by nasal administration of an LDL component is also attractive for clinical medicine.

[0107] In summary, the present disclosure provides fusion products and related compositions methods and systems that in several embodiments allow performing a strategy for atheroprotective immunization. A peptide sequence from apolipoprotein B-100 of low-density lipoprotein fused with a carrier such as the B subunit of cholera toxin is described and used for immunization of mice and in particular for intranasal immunization of Ape^{-/-} mice. Methods and systems herein described led to antigen-specific regulatory T cells and a 35% reduction of atherosclerosis.

[0108] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the fusion proteins, compositions, systems and methods of the disclosure, and are not intended to limit the scope of what the inventors regard as their disclosure. Modifications of the above-described modes for carrying out the disclosure that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the disclosure pertains.

[0109] It is to be understood that the disclosures are not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. The term "plurality" includes two or more referents unless the content clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0110] When a Markush group or other grouping is used herein, all individual members of the group and all combinations and possible subcombinations of the group are intended to be individually included in the disclosure. Every combination of components or materials described or exemplified herein can be used to practice the disclosure, unless otherwise stated. One of ordinary skill in the art will appreciate that methods, device elements, and materials other than those specifically exemplified can be employed in the practice of the disclosure without resort to undue experimentation. All art-known functional equivalents, of any such methods, device elements, and materials are intended to be included in this disclosure. Whenever a range is given in the specification, for example, a temperature range, a frequency range, a time range, or a composition range, all intermediate ranges and all subranges, as well as, all individual values included in the ranges given are intended to be included in the disclosure. Any one or more individual members of a range or group disclosed herein can be excluded from a claim of this disclosure. The disclosure illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein.

[0111] A number of embodiments of the disclosure have been described. The specific embodiments provided herein are examples of useful embodiments of the disclosure and it will be apparent to one skilled in the art that the disclosure can be carried out using a large number of variations of the fusion proteins, fusion protein components, compositions, methods steps, and systems set forth in the present description. As will be obvious to one of skill in the art, methods and devices useful for the present methods can include a large number of optional composition and processing elements and steps.

[0112] In particular, it will be understood that various modifications may be made without departing from the scope of the present disclosure. Accordingly, other embodiments are within the scope of the following claims.

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5	Leu	Gln	Glu	Leu												
				20												

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20	Arg	Ala	Asn	Leu												
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40	Ser	Asp	Glu	Ala												
				20												

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55	Ser	Ser	Pro	Ile												
				20												

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 30 Asn Pro Leu Leu
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 35 <210> 26
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 1 5 10 15
 50 Pro Glu Pro Ser
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 <210> 27
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 Arg Asp Gln Arg
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<400> 28

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 Val Asn Asn Tyr
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35 Ala Val Asn Asn Tyr His Lys Thr Asn Pro Thr Gly Thr Gln Glu Leu
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40 Leu Asp Ile Ala
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 Thr Gly Asp Glu
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 Gln Lys Ala Ala
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25 Pro Gly Asp Lys
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40 Pro Ser Gln Ala
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50 <220>
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<400> 37

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	Trp	Glu	Gln	Asn	Glu	Gln	Val	Lys	Asn	Phe	Val	Ala	Ser	His	Ile	Ala
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20	Asn	Ile	Leu	Asn
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40	Leu	Val	Lys	Glu
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55	Asp	Phe	Arg	Lys
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 1 5 10 15
 Lys Thr Thr Leu
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 Glu Ile Gly Leu
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20 Ile Glu Ile Gly Leu Glu Gly Lys Gly Phe Glu Pro Thr Leu Glu Ala
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25 Leu Phe Gly Lys
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30 <210> 46
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<400> 46

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 Leu Tyr Trp Val
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 Leu Val Asp His
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 Asp Met Val Asn
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 Asp Leu Lys Ser
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Leu Leu Gly Lys
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25 Ile Pro Gln Met
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Gly Ile Pro Gln Met Ile Gly Glu Val Ile Arg Lys Gly Ser Lys Asn
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40 Asp Phe Phe Leu
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<400> 54

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Asn Asp Phe Phe Leu His Tyr Ile Phe Met Glu Asn Ala Phe Glu Leu
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5 Pro Thr Gly Ala
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10 <212> PRT

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20 Leu Pro Thr Gly Ala Gly Leu Gln Leu Gln Ile Ser Ser Ser Gly Val
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Ile Ala Pro Gly
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40 Met Gln Ala Glu
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Asn Met Gln Ala Glu Leu Val Ala Lys Pro Ser Val Ser Val Glu Phe

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5 Val Thr Asn Met
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20 Val Gln Met Asn
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30 <223> Synthetic peptide

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His Val Ala Leu
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50 Ala His Val Ala Leu Lys Ala Gly Lys Leu Lys Phe Ile Ile Pro Ser
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55 Pro Lys Arg Pro
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<400> 61

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Leu	Val	Ser	Thr
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<400> 62

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His	Leu	Val	Ser	Thr	Thr	Lys	Thr	Glu	Val	Ile	Pro	Pro	Leu	Ile	Glu
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35

Asn	Arg	Gln	Ser
			20

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Glu	Asn	Arg	Gln	Ser	Trp	Ser	Val	Cys	Lys	Gln	Val	Phe	Pro	Gly	Leu
1				5					10					15	

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Asn	Tyr	Cys	Thr
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Ser Ala Ser Tyr

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<210> 65

$\langle 211 \rangle$ 20

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<223> Synthetic peptide

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Asp Ser Ala Ser Tyr Tyr Pro Leu Thr Gly Asp Thr Arg Leu Glu Leu
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Glu Leu Arg Pro
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30 <210> 66

<211> 20

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<400> 66

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Leu	Glu	Leu	Arg	Pro	Thr	Gly	Glu	Ile	Glu	Gln	Tyr	Ser	Val	Ser	Ala
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45 Thr Tyr Glu Leu
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<210> 67

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<223> Synthetic peptide

55 <400> 67

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Ala Thr Tyr Glu Leu Gln Arg Glu Asp Arg Ala Leu Val Asp Thr Leu
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5 Lys Phe Val Thr
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Leu Lys Phe Val Thr Gln Ala Glu Gly Ala Lys Gln Thr Glu Ala Thr
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20 Met Thr Phe Lys
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25 <210> 69

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Val Gln Ile Pro
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Glu Val Gln Ile Pro Asp Phe Asp Val Asp Leu Gly Thr Ile Leu Arg
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55 Val Asn Asp Glu
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 Leu Asp Ile Gln
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 His Leu Ser Cys
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 Gly His Leu Ser Cys Asp Thr Lys Glu Glu Arg Lys Ile Lys Gly Val
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 Ile Ser Ile Pro
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<400> 74

5 Val Ile Ser Ile Pro Arg Leu Gln Ala Glu Ala Arg Ser Glu Ile Leu
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Ala His Trp Ser
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Ala Thr Ala Tyr
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35 Ser Ala Thr Ala Tyr Gly Ser Thr Val Ser Lys Arg Val Ala Trp His
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40 Tyr Asp Glu Glu
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55 His Tyr Asp Glu Glu Lys Ile Glu Phe Glu Trp Asn Thr Gly Thr Asn
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Val Asp Thr Lys
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 His Arg Val Pro
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5 Ser Lys Leu Ile Val Ala Met Ser Ser Trp Leu Gln Lys Ala Ser Gly
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 Ser Leu Pro Tyr
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20 Gly Ser Leu Pro Tyr Thr Gln Thr Leu Gln Asp His Leu Asn Ser Leu
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25 Lys Glu Phe Asn
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40 Pro Glu Asn Leu
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Ile Pro Glu Asn Leu Phe Leu Lys Ser Asp Gly Arg Val Lys Tyr Thr
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5 Leu Asn Lys Asn
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Thr Leu Asn Lys Asn Ser Leu Lys Ile Glu Ile Pro Leu Pro Phe Gly
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Gly Lys Ser Ser
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Pro Ala Leu His
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Thr Pro Ala Leu His Phe Lys Ser Val Gly Phe His Leu Pro Ser Arg
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Glu Phe Gln Val
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 Ser Asn Leu Tyr
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Asp Ser Val Val
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Glu Thr Thr Tyr
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<210> 93

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Gly Glu Thr Thr Tyr Asp His Lys Asn Thr Phe Thr Leu Ser Cys Asp
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Gly Ser Leu Arg
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<210> 94

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<220>

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5 His Val Glu Lys
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10 <212> PRT

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<400> 95

Ser His Val Glu Lys Leu Gly Asn Asn Pro Val Ser Lys Gly Leu Leu
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Ile Phe Asp Ala
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25

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<220>

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Leu Ile Phe Asp Ala Ser Ser Ser Trp Gly Pro Gln Met Ser Ala Ser
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Val His Leu Asp
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Val Lys Ile Asp
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 Gly Thr Tyr Gly
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 Gly Thr Asn Gln
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25 Ala Ser Leu Lys
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 Thr Ala Ser Leu Lys Tyr Glu Asn Tyr Glu Leu Thr Leu Lys Ser Asp
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40 Thr Asn Gly Lys
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50 <400> 107

Ser Leu Asn Ser His Gly Leu Glu Leu Asn Ala Asp Ile Leu Gly Thr
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55 Asp Lys Ile Asn
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Asn Ala Lys Phe
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 Lys Leu Asp Asn
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<400> 118

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Ser Asp Leu Lys
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Gly Ala Tyr Gln
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<223> Synthetic peptide

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1 5 10 15

5 Ser Ala Ala Leu
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Ser Ser Ala Ala Leu Ser Ala Ser Tyr Lys Ala Asp Thr Val Ala Lys

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Val Gln Gly Val
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40 Gly Leu Ala Ser
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Gln Asp Leu Asp
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Ser Gln Asp Leu Asp Ala Tyr Asn Thr Lys Asp Lys Ile Gly Val Glu
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Leu Thr Gly Arg
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Ile Lys Val Pro
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<400> 134

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15 <400> 135

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20 Ile Val Ala Phe
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 Leu Lys His Ile
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 Ala Leu Gly Lys
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 Ala Leu Thr Lys
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5 Tyr Asp Leu His
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20 Glu Ile Ile Glu
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40 Arg Val Asn Leu
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55 Glu Asn Ile Asp
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Arg Ala Lys Val His Glu Leu Ile Glu Arg Tyr Glu Val Asp Gln Gln
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Ile Gln Val Leu
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Lys Leu Lys Glu
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Tyr Lys Leu Lys Glu Thr Ile Gln Lys Leu Ser Asn Val Leu Gln Gln
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Val Lys Ile Lys
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 Asp Ala Val Lys
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 Arg Glu Val Thr
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40 Thr Arg Asp Arg
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50 <400> 167

Asp Thr Arg Asp Arg Met Tyr Gln Met Asp Ile Gln Gln Glu Leu Gln
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 Phe Ala Glu Gln
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 Ala Leu Val Glu
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 Leu Gly Thr Met
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45 <210> 174
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 Gly Ile Ala Ala Ser Ile Thr Ala Lys Gly Glu Ser Lys Leu Glu Val
 1 5 10 15
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 Leu Asn Phe Asp
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 Val Leu Asn Phe Asp Phe Gln Ala Asn Ala Gln Leu Ser Asn Pro Lys
 1 5 10 15
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 Ile Asn Pro Leu
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 45
 Lys Ile Asn Pro Leu Ala Leu Lys Glu Ser Val Lys Phe Ser Ser Lys
 1 5 10 15
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 Tyr Leu Arg Thr
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 <210> 188
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<220>

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<400> 188

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Lys Tyr Leu Arg Thr Glu His Gly Ser Glu Met Leu Phe Phe Gly Asn
1 5 10 15

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Ala Ile Glu Gly
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<210> 189

<211> 20

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<212> PRT

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<220>

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<400> 189

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Asn Ala Ile Glu Gly Lys Ser Asn Thr Val Ala Ser Leu His Thr Glu
1 5 10 15

Lys Asn Thr Leu
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<210> 190

<211> 20

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<220>

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<400> 190

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Glu Lys Asn Thr Leu Glu Leu Ser Asn Gly Val Ile Val Lys Ile Asn
1 5 10 15

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Asn Gln Leu Thr
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<211> 20

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<213> Artificial Sequence

<220>

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<400> 191

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Asn Asn Gln Leu Thr Leu Asp Ser Asn Thr Lys Tyr Phe His Lys Leu
1 5 10 15

5 Asn Ile Pro Lys
20

<210> 192

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10 <212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

15 <400> 192

Leu Asn Ile Pro Lys Leu Asp Phe Ser Ser Gln Ala Asp Leu Arg Asn
1 5 10 15

20 Glu Ile Lys Thr
20

25 <210> 193

<211> 20

<212> PRT

<213> Artificial Sequence

30 <220>

<223> Synthetic peptide

<400> 193

35 Asn Glu Ile Lys Thr Leu Leu Lys Ala Gly His Ile Ala Trp Thr Ser
1 5 10 15

40 Ser Gly Lys Gly
20

<210> 194

<211> 20

<212> PRT

45 <213> Artificial Sequence

<220>

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50 <400> 194

Ser Ser Gly Lys Gly Ser Trp Lys Trp Ala Cys Pro Arg Phe Ser Asp
1 5 10 15

55 Glu Gly Thr His

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10	<220>	
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	Leu Thr Ser Phe	
20	20	
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30	<400> 196	
	Pro Leu Thr Ser Phe Gly Leu Ser Asn Lys Ile Asn Ser Lys His Leu	
	1 5 10 15	
35	Arg Val Asn Gln	
	20	
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40	<211> 20	
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45	<223> Synthetic peptide	
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	1 5 10 15	
	Ser Lys Leu Glu	
	20	
55	<210> 198	
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<213> Artificial Sequence

<220>

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<400> 198

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Phe Ser Lys Leu Glu Ile Gln Ser Gln Val Asp Ser Gln His Val Gly
1 5 10 15

His Ser Val Leu
20

15

<210> 199

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<213> Artificial Sequence

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<220>

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<400> 199

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Gly His Ser Val Leu Thr Ala Lys Gly Met Ala Leu Phe Gly Glu Gly
1 5 10 15

Lys Ala Glu Phe
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<210> 200

<211> 20

<212> PRT

35

<213> Artificial Sequence

<220>

<223> Synthetic peptide

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<400> 200

Gly Lys Ala Glu Phe Thr Gly Arg His Asp Ala His Leu Asn Gly Lys
1 5 10 15

45

Val Ile Gly Thr
20

50

<210> 201

<211> 20

<212> PRT

<213> Artificial Sequence

55

<220>

<223> Synthetic peptide

<400> 201

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	Lys	Val	Ile	Gly	Thr	Leu	Lys	Asn	Ser	Leu	Phe	Phe	Ser	Ala	Gln	Pro
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5	Phe	Glu	Ile	Thr
				20

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15	<400>	202

	Pro	Phe	Glu	Ile	Thr	Ala	Ser	Thr	Asn	Asn	Glu	Gly	Asn	Leu	Lys	Val
	1				5					10					15	

20	Arg	Phe	Pro	Leu
				20

25	<210>	203
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	<213>	Artificial Sequence
30	<220>	
	<223>	Synthetic peptide
	<400>	203

35	Val	Arg	Phe	Pro	Leu	Arg	Leu	Thr	Gly	Lys	Ile	Asp	Phe	Leu	Asn	Asn
	1				5					10					15	

40	Tyr	Ala	Leu	Phe
				20

	<210>	204
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	<223>	Synthetic peptide
50	<400>	204

	Asn	Tyr	Ala	Leu	Phe	Leu	Ser	Pro	Ser	Ala	Gln	Gln	Ala	Ser	Trp	Gln
	1				5					10					15	

55	Val	Ser	Ala	Arg
				20

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 Gln Val Ser Ala Arg Phe Asn Gln Tyr Lys Tyr Asn Gln Asn Phe Ser
 1 5 10 15
 Ala Gly Asn Asn
 15 20
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 25
 Ser Ala Gly Asn Asn Glu Asn Ile Met Glu Ala His Val Gly Ile Asn
 1 5 10 15
 Gly Glu Ala Asn
 30 20
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 35
 <220>
 <223> Synthetic peptide
 40
 <400> 207
 45
 Asn Gly Glu Ala Asn Leu Asp Phe Leu Asn Ile Pro Leu Thr Ile Pro
 1 5 10 15
 Glu Met Arg Leu
 50 20
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 55
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<400> 208

5 Pro Glu Met Arg Leu Pro Tyr Thr Ile Ile Thr Thr Pro Pro Leu Lys
 1 5 10 15

 Asp Phe Ser Leu
 20

10 <210> 209
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 <212> PRT
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15 <220>
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<400> 209

20 Lys Asp Phe Ser Leu Trp Glu Lys Thr Gly Leu Lys Glu Phe Leu Lys
 1 5 10 15

25 Thr Thr Lys Gln
 20

 <210> 210
 <211> 20
 <212> PRT
30 <213> Artificial Sequence

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35 <400> 210

 Lys Thr Thr Lys Gln Ser Phe Asp Leu Ser Val Lys Ala Gln Tyr Lys
 1 5 10 15

40 Lys Asn Lys His
 20

45 <210> 211
 <211> 20
 <212> PRT
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50 <220>
 <223> Synthetic peptide

<400> 211

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Lys Lys Asn Lys His Arg His Ser Ile Thr Asn Pro Leu Ala Val Leu
 1 5 10 15

 5 Cys Glu Phe Ile
 20

 <210> 212
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 10 <212> PRT
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 15
 <400> 212

 Leu Cys Glu Phe Ile Ser Gln Ser Ile Lys Ser Phe Asp Arg His Phe
 1 5 10 15
 20

 Glu Lys Asn Arg
 20

 25 <210> 213
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 <212> PRT
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 30 <220>
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 <400> 213

 35 Phe Glu Lys Asn Arg Asn Asn Ala Leu Asp Phe Val Thr Lys Ser Tyr
 1 5 10 15

 Asn Glu Thr Lys
 20
 40

 <210> 214
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 45 <213> Artificial Sequence

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 50 <400> 214

 Tyr Asn Glu Thr Lys Ile Lys Phe Asp Lys Tyr Lys Ala Glu Lys Ser
 1 5 10 15
 55

 His Asp Glu Leu
 20

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<210> 215
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 1 5 10 15
 Pro Val Val Asn
 20
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 20
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 25
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 Val Pro Val Val Asn Val Glu Val Ser Pro Phe Thr Ile Glu Met Ser
 1 5 10 15
 30
 Ala Phe Gly Tyr
 20
 35
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 40
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 45
 Ser Ala Phe Gly Tyr Val Phe Pro Lys Ala Val Ser Met Pro Ser Phe
 1 5 10 15
 Ser Ile Leu Gly
 20
 50
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<400> 218

5 Phe Ser Ile Leu Gly Ser Asp Val Arg Val Pro Ser Tyr Thr Leu Ile
1 5 10 15

Leu Pro Ser Leu
20

10 <210> 219
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15 <220>
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<400> 219

20 Ile Leu Pro Ser Leu Glu Leu Pro Val Leu His Val Pro Arg Asn Leu
1 5 10 15

25 Lys Leu Ser Leu
20

<210> 220
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30 <213> Artificial Sequence

<220>
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35 <400> 220

Leu Lys Leu Ser Leu Pro His Phe Lys Glu Leu Cys Thr Ile Ser His
1 5 10 15

40 Ile Phe Ile Pro
20

45 <210> 221
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50 <220>
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<400> 221

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	His	Ile	Phe	Ile	Pro	Ala	Met	Gly	Asn	Ile	Thr	Tyr	Asp	Phe	Ser	Phe
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5	Lys	Ser	Ser	Val
				20

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15	<400>	222

	Phe	Lys	Ser	Ser	Val	Ile	Thr	Leu	Asn	Thr	Asn	Ala	Glu	Leu	Phe	Asn
	1				5					10					15	

20	Gln	Ser	Asp	Ile
				20

25	<210>	223
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	<223>	Synthetic peptide
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35	Asn	Gln	Ser	Asp	Ile	Val	Ala	His	Leu	Leu	Ser	Ser	Ser	Ser	Ser	Val
	1				5					10					15	

	Ile	Asp	Ala	Leu
				20

40	<210>	224
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45	<220>	
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50	Val	Ile	Asp	Ala	Leu	Gln	Tyr	Lys	Leu	Glu	Gly	Thr	Thr	Arg	Leu	Thr
	1				5					10					15	

55	Arg	Lys	Arg	Gly
				20

	<210>	225
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5 <220>
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<400> 225

10 Thr Arg Lys Arg Gly Leu Lys Leu Ala Thr Ala Leu Ser Leu Ser Asn
 1 5 10 15

15 Lys Phe Val Glu
 20

<210> 226
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 20 <213> Artificial Sequence

<220>
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25 <400> 226

Asn Lys Phe Val Glu Gly Ser His Asn Ser Thr Val Ser Leu Thr Thr
 1 5 10 15

30 Lys Asn Met Glu
 20

<210> 227
 35 <211> 20
 <212> PRT
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<220>
 40 <223> Synthetic peptide

<400> 227

45 Thr Lys Asn Met Glu Val Ser Val Ala Lys Thr Thr Lys Ala Glu Ile
 1 5 10 15

50 Pro Ile Leu Arg
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<210> 228
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55 <220>
 <223> Synthetic peptide

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<400> 228

5 Ile Pro Ile Leu Arg Met Asn Phe Lys Gln Glu Leu Asn Gly Asn Thr
1 5 10 15

Lys Ser Lys Pro
20

10 <210> 229

<211> 20

<212> PRT

<213> Artificial Sequence

15 <220>

<223> Synthetic peptide

<400> 229

20 Thr Lys Ser Lys Pro Thr Val Ser Ser Ser Met Glu Phe Lys Tyr Asp
1 5 10 15

25 Phe Asn Ser Ser
20

<210> 230

<211> 20

<212> PRT

30 <213> Artificial Sequence

<220>

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35 <400> 230

Asp Phe Asn Ser Ser Met Leu Tyr Ser Thr Ala Lys Gly Ala Val Asp
1 5 10 15

40 His Lys Leu Ser
20

<210> 231

45 <211> 20

<212> PRT

<213> Artificial Sequence

<220>

50 <223> Synthetic peptide

<400> 231

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	Asp	His	Lys	Leu	Ser	Leu	Glu	Ser	Leu	Thr	Ser	Tyr	Phe	Ser	Ile	Glu
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5	Ser	Ser	Thr	Lys
				20

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	<223>	Synthetic peptide
15	<400>	232

	Glu	Ser	Ser	Thr	Lys	Gly	Asp	Val	Lys	Gly	Ser	Val	Leu	Ser	Arg	Glu
	1				5					10					15	

20	Tyr	Ser	Gly	Thr
				20

25	<210>	233
	<211>	20
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	<213>	Artificial Sequence
30	<220>	
	<223>	Synthetic peptide
	<400>	233

35	Glu	Tyr	Ser	Gly	Thr	Ile	Ala	Ser	Glu	Ala	Asn	Thr	Tyr	Leu	Asn	Ser
	1				5					10					15	

40	Lys	Ser	Thr	Arg
				20

	<210>	234
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45	<213>	Artificial Sequence
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	<223>	Synthetic peptide
50	<400>	234

	Ser	Lys	Ser	Thr	Arg	Ser	Ser	Val	Lys	Leu	Gln	Gly	Thr	Ser	Lys	Ile
	1				5					10					15	

55	Asp	Asp	Ile	Trp
				20

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 Ile Asp Asp Ile Trp Asn Leu Glu Val Lys Glu Asn Phe Ala Gly Glu
 1 5 10 15
 15
 Ala Thr Leu Gln
 20
 <210> 236
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 20
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 25
 <400> 236
 30
 Glu Ala Thr Leu Gln Arg Ile Tyr Ser Leu Trp Glu His Ser Thr Lys
 1 5 10 15
 35
 Asn His Leu Gln
 20
 40
 <210> 237
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 45
 <220>
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 <400> 237
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 Lys Asn His Leu Gln Leu Glu Gly Leu Phe Phe Thr Asn Gly Glu His
 1 5 10 15
 55
 Thr Ser Lys Ala
 20
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<400> 238

5 His Thr Ser Lys Ala Thr Leu Glu Leu Ser Pro Trp Gln Met Ser Ala
 1 5 10 15

 Leu Val Gln Val
 20

10 <210> 239
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 <212> PRT
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15 <220>
 <223> Synthetic peptide

<400> 239

20 Ala Leu Val Gln Val His Ala Ser Gln Pro Ser Ser Phe His Asp Phe
 1 5 10 15

 Pro Asp Leu Gly
 20

25 <210> 240
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 <212> PRT
30 <213> Artificial Sequence

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35 <400> 240

 Phe Pro Asp Leu Gly Gln Glu Val Ala Leu Asn Ala Asn Thr Lys Asn
 1 5 10 15

40 Gln Lys Ile Arg
 20

45 <210> 241
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50 <223> Synthetic peptide

<400> 241

55 Asn Gln Lys Ile Arg Trp Lys Asn Glu Val Arg Ile His Ser Gly Ser
 1 5 10 15

 Phe Gln Ser Gln
 20

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<210> 242
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<220>
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<400> 242

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Ser Phe Gln Ser Gln Val Glu Leu Ser Asn Asp Gln Glu Lys Ala His
 1 5 10 15

15

Leu Asp Ile Ala
 20

<210> 243
 <211> 20
 <212> PRT
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20

<220>
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25

<400> 243

His Leu Asp Ile Ala Gly Ser Leu Glu Gly His Leu Arg Phe Leu Lys
 1 5 10 15

30

Asn Ile Ile Leu
 20

<210> 244
 <211> 20
 <212> PRT
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35

<220>
 <223> Synthetic peptide

40

<400> 244

Lys Asn Ile Ile Leu Pro Val Tyr Asp Lys Ser Leu Trp Asp Phe Leu
 1 5 10 15

45

Lys Leu Asp Val
 20

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<210> 245
 <211> 20
 <212> PRT
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55

<220>
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<400> 245

5 Leu Lys Leu Asp Val Thr Thr Ser Ile Gly Arg Arg Gln His Leu Arg
 1 5 10 15

 Val Ser Thr Ala
 20

10 <210> 246

 <211> 20

 <212> PRT

 <213> Artificial Sequence

15 <220>

 <223> Synthetic peptide

 <400> 246

20 Arg Val Ser Thr Ala Phe Val Tyr Thr Lys Asn Pro Asn Gly Tyr Ser
 1 5 10 15

25 Phe Ser Ile Pro
 20

 <210> 247

 <211> 20

 <212> PRT

30 <213> Artificial Sequence

 <220>

 <223> Synthetic peptide

35 <400> 247

 Ser Phe Ser Ile Pro Val Lys Val Leu Ala Asp Lys Phe Ile Thr Pro
 1 5 10 15

40 Gly Leu Lys Leu
 20

 <210> 248

45 <211> 20

 <212> PRT

 <213> Artificial Sequence

 <220>

50 <223> Synthetic peptide

 <400> 248

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	Pro	Gly	Leu	Lys	Leu	Asn	Asp	Leu	Asn	Ser	Val	Leu	Val	Met	Pro	Thr
	1				5					10					15	

5	Phe	His	Val	Pro
				20

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10	<212>	PRT
	<213>	Artificial Sequence
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	<223>	Synthetic peptide
15	<400>	249

	Thr	Phe	His	Val	Pro	Phe	Thr	Asp	Leu	Gln	Val	Pro	Ser	Cys	Lys	Leu
20	1				5					10					15	

	Asp	Phe	Arg	Glu
				20

25	<210>	250
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	<213>	Artificial Sequence
30	<220>	
	<223>	Synthetic peptide
	<400>	250

35	Leu	Asp	Phe	Arg	Glu	Ile	Gln	Ile	Tyr	Lys	Lys	Leu	Arg	Thr	Ser	Ser
	1				5					10					15	

40	Phe	Ala	Leu	Asn
				20

	<210>	251
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45	<213>	Artificial Sequence
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	<223>	Synthetic peptide
50	<400>	251

	Ser	Phe	Ala	Leu	Asn	Leu	Pro	Thr	Leu	Pro	Glu	Val	Lys	Phe	Pro	Glu
	1				5					10					15	

55	Val	Asp	Val	Leu
				20

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<220>
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Glu Val Asp Val Leu Thr Lys Tyr Ser Gln Pro Glu Asp Ser Leu Ile

15

1 5 10 15

Pro Phe Phe Glu
 20

20

<210> 253
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25

<220>
 <223> Synthetic peptide

<400> 253

30

Ile Pro Phe Phe Glu Ile Thr Val Pro Glu Ser Gln Leu Thr Val Ser
 1 5 10 15

35

Gln Phe Thr Leu
 20

<210> 254
 <211> 20
 <212> PRT
 <213> Artificial Sequence

40

<220>
 <223> Synthetic peptide

45

<400> 254

Ser Gln Phe Thr Leu Pro Lys Ser Val Ser Asp Gly Ile Ala Ala Leu
 1 5 10 15

50

Asp Leu Asn Ala
 20

55

<210> 255
 <211> 20
 <212> PRT
 <213> Artificial Sequence

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<220>

<223> Synthetic peptide

<400> 255

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Leu Asp Leu Asn Ala Val Ala Asn Lys Ile Ala Asp Phe Glu Leu Pro
1 5 10 15

10

Thr Ile Ile Val
20

<210> 256

<211> 20

15

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

20

<400> 256

Pro Thr Ile Ile Val Pro Glu Gln Thr Ile Glu Ile Pro Ser Ile Lys
1 5 10 15

25

Phe Ser Val Pro
20

30

<210> 257

<211> 20

<212> PRT

<213> Artificial Sequence

35

<220>

<223> Synthetic peptide

<400> 257

40

Lys Phe Ser Val Pro Ala Gly Ile Val Ile Pro Ser Phe Gln Ala Leu
1 5 10 15

45

Thr Ala Arg Phe
20

<210> 258

<211> 20

<212> PRT

50

<213> Artificial Sequence

<220>

<223> Synthetic peptide

55

<400> 258

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Leu Thr Ala Arg Phe Glu Val Asp Ser Pro Val Tyr Asn Ala Thr Trp
1 5 10 15

5 Ser Ala Ser Leu
20

<210> 259

<211> 20

10 <212> PRT

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<220>

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15 <400> 259

Trp Ser Ala Ser Leu Lys Asn Lys Ala Asp Tyr Val Glu Thr Val Leu
1 5 10 15

20 Asp Ser Thr Cys

25 20

<210> 260

<211> 20

30 <212> PRT

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35 <400> 260

Leu Asp Ser Thr Cys Ser Ser Thr Val Gln Phe Leu Glu Tyr Glu Leu
1 5 10 15

40 Asn Val Leu Gly
20

45 <210> 261

<211> 20

<212> PRT

<213> Artificial Sequence

50 <220>

<223> Synthetic peptide

<400> 261

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Leu Asn Val Leu Gly Thr His Lys Ile Glu Asp Gly Thr Leu Ala Ser
 1 5 10 15

 5 Lys Thr Lys Gly
 20

 <210> 262
 <211> 20
 10 <212> PRT
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 15
 <400> 262

 Ser Lys Thr Lys Gly Thr Leu Ala His Arg Asp Phe Ser Ala Glu Tyr
 1 5 10 15
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 Glu Glu Asp Gly
 20

 25 <210> 263
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 <212> PRT
 <213> Artificial Sequence

 30 <220>
 <223> Synthetic peptide

 <400> 263

 35 Tyr Glu Glu Asp Gly Lys Phe Glu Gly Leu Gln Glu Trp Glu Gly Lys
 1 5 10 15

 Ala His Leu Asn
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 40

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 55 Arg Tyr Gln Lys
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 Pro Ala Val Gly
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	Glu Glu Leu Cys
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	Ser Tyr Phe Gln
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 Gln Tyr Ile Met
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20	Leu	Ser	Ala	Thr
				20

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	<211>	20
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	<213>	Artificial Sequence
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	1				5					10					15	

40	Thr	Lys	Lys	Ile
				20

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	Thr	Lys	Lys	Ile	Ile	Ser	Asp	Tyr	His	Gln	Gln	Phe	Arg	Tyr	Lys	Leu
	1				5					10					15	

55	Gln	Asp	Phe	Ser
				20

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 Ala Glu Ser Lys
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 Thr Phe Leu Ile
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10

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cta 63

25

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ttgtgattcg a 71

40

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45 <220>
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<400> 305

⁵⁰

Lys	Thr	Thr	Lys	Gln	Ser	Phe	Asp	Leu	Ser	Val	Lys	Ala	Gln	Tyr	Lys
1				5					10					15	

55 Lys Asn Ser Asp Lys His
20

Claims

1. A fusion protein comprising
an antigenic fragment of apoB-100 protein, or a derivative thereof, and
subunit B of cholera toxin as a protein carrier,
wherein the antigenic fragment of apoB-100 or derivative thereof and the protein carrier are linked in the fusion
protein in a 1:1 fragment:carrier molar ratio; and
wherein the fusion protein is capable of inducing antigen specific regulatory T cells, the antigen specific regulatory
T cells being specific for the antigenic fragment of apoB-100 or derivative thereof,
for use to treat and/or prevent atherosclerosis in an individual,
wherein the fusion protein is for mucosal administration to the individual in an amount effective to elicit an antigen
specific Treg immunomodulatory response specific for the antigenic fragment of apoB-100 protein, or derivative
thereof, in the individual,
and wherein a derivative of an antigenic fragment of apoB-100 protein indicates a polypeptide structurally related
to the antigenic fragment of apoB-100 and derivable from the antigenic fragment of apoB-100 by a modification that
introduces a feature that is not present in the antigenic fragment of apoB-100, while retaining one or more antigenic
properties of apoB-100 or the antigenic fragment of apoB-100.
2. The fusion protein for use according to claim 1, wherein the antigenic fragment of apoB-100 comprises one or more
peptide selected from the group consisting of P2, P45, P102 and P210 of table 1.
3. The fusion protein for use according to claim 1, wherein the antigenic fragment of apoB-100 comprises P210 of table 1.
4. An immunogenic composition comprising the fusion protein for use according to any one of claims 1 to 3 together
with an adjuvant and/or a pharmaceutically acceptable excipient.
5. A method to produce a fusion protein, the method comprising
attaching an antigenic fragment of apoB-100, or a derivative thereof, to a protein carrier in a 1:1 fragment:carrier
molar ratio to provide a fusion protein capable of inducing antigen specific regulatory T cells, wherein the protein
carrier is subunit B of cholera toxin,
the antigen specific regulatory T cells being specific for the fragment of apoB-100 or the derivative thereof,
wherein a derivative of an antigenic fragment of apoB-100 protein indicates a polypeptide structurally related to the
antigenic fragment of apoB-100 and derivable from the antigenic fragment of apoB-100 by a modification that
introduces a feature that is not present in the antigenic fragment of apoB-100, while retaining one or more antigenic
properties of apoB-100 or the antigenic fragment of apoB-100.
6. The method of claim 5, wherein the antigenic fragment of apoB-100 comprises one or more of P2, P45, P102, and
P210 of table 1.
7. The method of any one of claims 5 or 6, wherein the attaching is performed by biological genetic engineering or by
chemical covalent conjugation.
8. *An in vitro* method to induce an antigen specific Treg cell, the method comprising
contacting a Treg cell with the fusion protein for use according to any one of claims 1 to 3, the contacting performed
for a time and under conditions to allow induction of a Treg response,
wherein the contacting results in the induction of an antigen-specific immune response specific for the fragment of
apoB-100, or derivative thereof, comprised in the fusion protein.

Patentansprüche

1. Fusionsprotein, umfassend:
ein antigenes Fragment von apoB-100-Protein oder ein Derivat davon, und Untereinheit B von Choleratoxin als
Proteinträger,
wobei das antigene Fragment von apoB-100-Protein oder eines Derivats davon und der Proteinträger in dem
Fusionsprotein in einem Fragment:Träger-Molverhältnis von 1:1 verknüpft sind; und
wobei das Fusionsprotein antigenspezifische regulatorische T-Zellen induzieren kann, wobei die antigenspe-

zifischen regulatorischen T-Zellen spezifisch für das antigene Fragment von apoB-100 oder das Derivat davon sind,

zur Verwendung bei der Behandlung und/oder Prävention von Atherosklerose in einem Individuum, wobei das Fusionsprotein über die Schleimhäute an das Individuum in einer Menge verabreicht wird, die wirksam eine antigenspezifische Tregimmunmodulatorische Reaktion, die für das antigene Fragment von apoB-100-Protein oder das Derivat davon spezifisch ist, in dem Individuum auslöst, und wobei ein Derivat eines antigenen Fragments von apoB-100-Protein ein Polypeptid anzeigt, das strukturell mit dem antigenen Fragment von apoB-100 verwandt ist, und sich von dem antigenen Fragment von apoB-100 durch eine Modifikation ableiten lässt, die ein Merkmal einführt, das in dem antigenen Fragment von apoB-100 nicht vorhanden ist, es aber trotzdem ein oder mehr antigene Eigenschaften von apoB-100 oder des antigenen Fragments von apoB-100 beibehält.

2. Fusionsprotein zur Verwendung nach Anspruch 1, wobei das antigene Fragment von apoB-100 ein oder mehr Peptide, ausgewählt aus der Gruppe, bestehend aus P2, P45, P102, und P210 von Tabelle 1, umfasst.

3. Fusionsprotein zur Verwendung nach Anspruch 1, wobei das antigene Fragment von apoB-100 P210 von Tabelle 1 umfasst.

4. Immunogene Zusammensetzung, umfassend das Fusionsprotein zur Verwendung nach einem der Ansprüche 1 bis 3 zusammen mit einem Hilfsstoff und/oder einem pharmazeutisch akzeptablen Exzipienten.

5. Verfahren zur Produktion eines Fusionsproteins, wobei das Verfahren folgendes umfasst:

Binden eines antigenen Fragments von apoB-100 oder eines Derivats davon, an einen Proteinträger in einem Fragment:Träger-Molverhältnis von 1:1, so dass ein Fusionsprotein bereitgestellt wird, das antigenspezifische regulatorische T-Zellen induzieren kann, wobei der Proteinträger Untereinheit B von Cholera toxin ist, wobei die antigenspezifischen regulatorischen T-Zellen spezifisch für das Fragment von apoB-100 oder das Derivat davon sind, wobei ein Derivat eines antigenen Fragments von apoB-100-Protein ein Polypeptid anzeigt, das strukturell mit dem antigenen Fragment von apoB-100 verwandt ist, und sich von dem antigenen Fragment von apoB-100 durch eine Modifikation ableiten lässt, die ein Merkmal einführt, das in dem antigenen Fragment von apoB-100 nicht vorhanden ist, es aber trotzdem ein oder mehr antigene Eigenschaften von apoB-100 oder dem antigenen Fragment von apoB-100 beibehält.

6. Verfahren nach Anspruch 5, wobei das antigene Fragment von apoB-100 ein oder mehr von P2, P45, P102, und P210 von Tabelle 1 umfasst.

7. Verfahren nach einem der Ansprüche 5 oder 6, wobei die Bindung durch biologische Genmanipulation oder durch chemische kovalente Konjugation erfolgt.

8. *In-vitro*-Verfahren zur Induktion einer antigenspezifischen Treg-Zelle, wobei das Verfahren das Zusammenbringen einer Treg-Zelle mit dem Fusionsprotein umfasst, zur Verwendung nach einem der Ansprüche 1 bis 3, wobei das Zusammenbringen hinreichend lange und unter Bedingungen erfolgt, dass die Induktion einer Treg-Reaktion ermöglicht wird, wobei das Zusammenbringen zur Induktion einer antigenspezifischen Immunreaktion führt, die spezifisch für das im Fusionsprotein enthaltene Fragment von apoB-100 oder das Derivat davon ist.

Revendications

1. Protéine de fusion, comprenant un fragment antigénique de la protéine apoB-100, ou un dérivé de celui-ci, et la sous-unité B de la toxine cholérique, en tant que protéine porteuse, dans laquelle le fragment antigénique de l'apoB-100, ou le dérivé de celui-ci, et la protéine porteuse, sont liés dans la protéine de fusion selon un rapport en moles fragment:porteuse de 1:1 ; et dans laquelle la protéine de fusion est capable d'induire des cellules T régulatrices spécifiques d'antigène, les cellules T régulatrices spécifiques d'antigène étant spécifiques du fragment antigénique de l'apoB-100 ou du dérivé

de celui-ci,

pour une utilisation pour traiter et/ou prévenir l'athérosclérose chez un individu,

la protéine de fusion étant destinée à une administration muqueuse à l'individu en une quantité efficace pour déclencher une réponse immunomodulatrice des Treg spécifique d'antigène, spécifique du fragment antigénique de la protéine apoB-100, ou du dérivé de celui-ci, chez l'individu,

et dans laquelle un dérivé d'un fragment antigénique de la protéine apoB-100 est indicatif d'un polypeptide qui d'un point de vue structural est associé au fragment antigénique de l'apoB-100 et peut dériver du fragment antigénique de l'apoB-100 par une modification qui introduit une particularité qui n'est pas présente dans le fragment antigénique de l'apoB-100, tout en conservant une ou plusieurs propriétés antigéniques de l'apoB-100 ou du fragment antigénique de l'apoB-100.

2. Protéine de fusion pour l'utilisation selon la revendication 1, dans laquelle le fragment antigénique de l'apoB-100 comprend un ou plusieurs peptides choisis dans le groupe consistant en P2, P45, P102 et P210 du tableau 1.

3. Protéine de fusion pour l'utilisation selon la revendication 1, dans laquelle le fragment antigénique de l'apoB-100 comprend P210 du tableau 1.

4. Composition immunogène comprenant la protéine de fusion pour l'utilisation selon l'une quelconque des revendications 1 à 3, en même temps qu'un adjuvant et/ou un excipient pharmaceutiquement acceptable.

5. Procédé de production d'une protéine de fusion, le procédé comprenant l'attachement d'un fragment antigénique de l'apoB-100, ou d'un dérivé de celui-ci, à une protéine porteuse selon un rapport en moles fragment:porteuse de 1:1, pour fournir une protéine de fusion capable d'induire des cellules T régulatrices spécifiques d'antigène, la protéine porteuse étant la sous-unité B de la toxine cholérique, les cellules T régulatrices spécifiques d'antigène étant spécifiques du fragment de l'apoB-100 ou du dérivé de celui-ci, dans lequel un dérivé d'un fragment antigénique de la protéine apoB-100 indique un polypeptide qui d'un point de vue structural est associé au fragment antigénique de l'apoB-100 et pouvant dériver du fragment antigénique de l'apoB-100 par une modification qui introduit une particularité qui n'est pas présente dans le fragment antigénique de l'apoB-100 tout en conservant une ou plusieurs propriétés antigéniques de l'apoB-100 ou du fragment antigénique de l'apoB-100.

6. Procédé selon la revendication 5, dans lequel le fragment antigénique de l'apoB-100 comprend un ou plusieurs de P2, P45, P102 et P210 du tableau 1.

7. Procédé selon l'une quelconque des revendications 5 ou 6, dans lequel l'attachement est réalisé par génie génétique biologique ou par conjugaison covalente chimique.

8. Procédé *in vitro* pour induire une cellule Treg spécifique d'antigène, le procédé comprenant :

la mise en contact d'une cellule Treg avec la protéine de fusion pour l'utilisation selon l'une quelconque des revendications 1 à 3, la mise en contact étant réalisée pendant un laps de temps et dans des conditions permettant l'induction d'une réponse Treg,

dans lequel la mise en contact conduit à l'induction d'une réponse immune spécifique d'antigène, spécifique du fragment de l'apoB-100 ou du dérivé de celui-ci, compris dans la protéine de fusion.

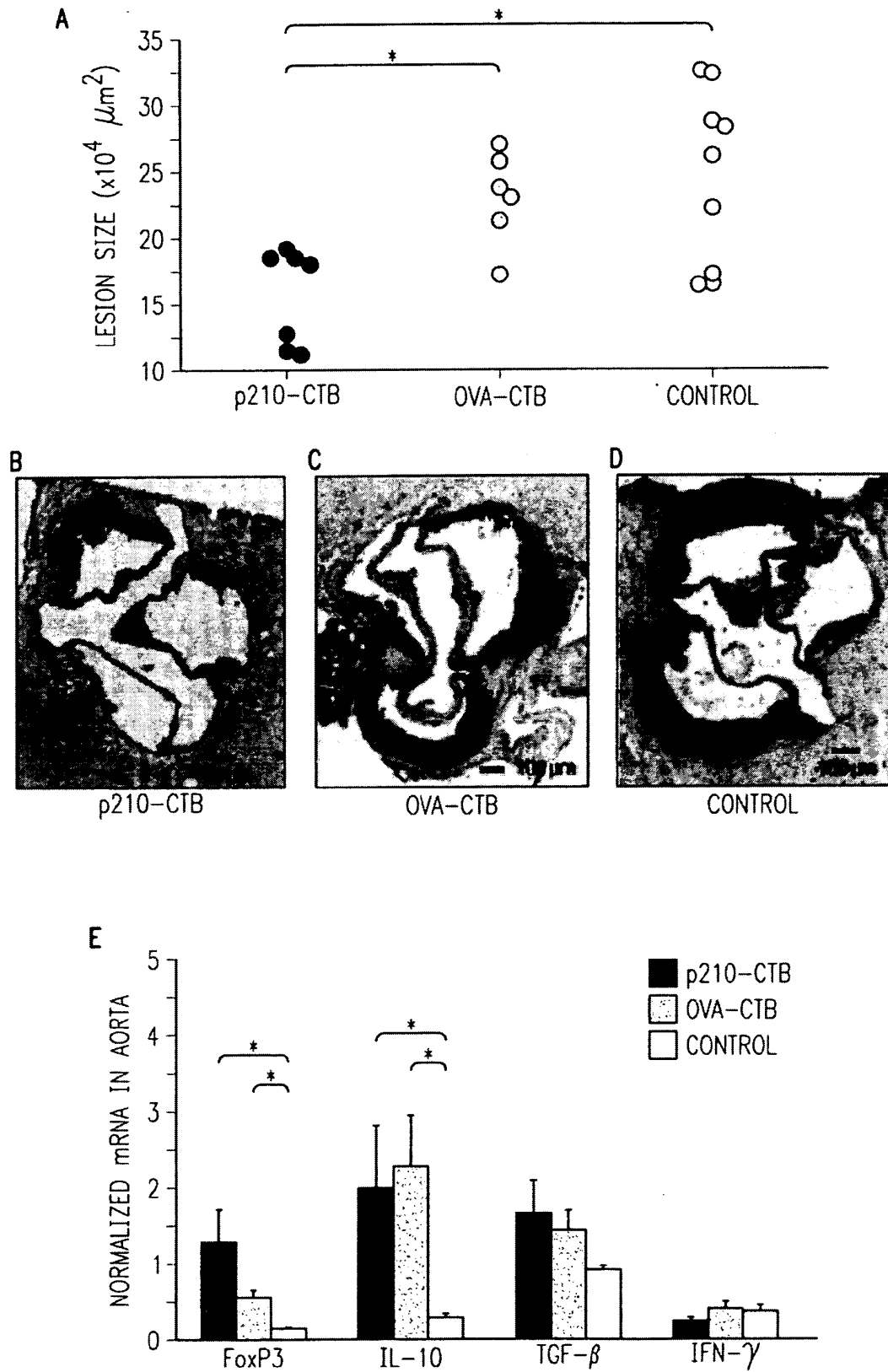


FIG. 1

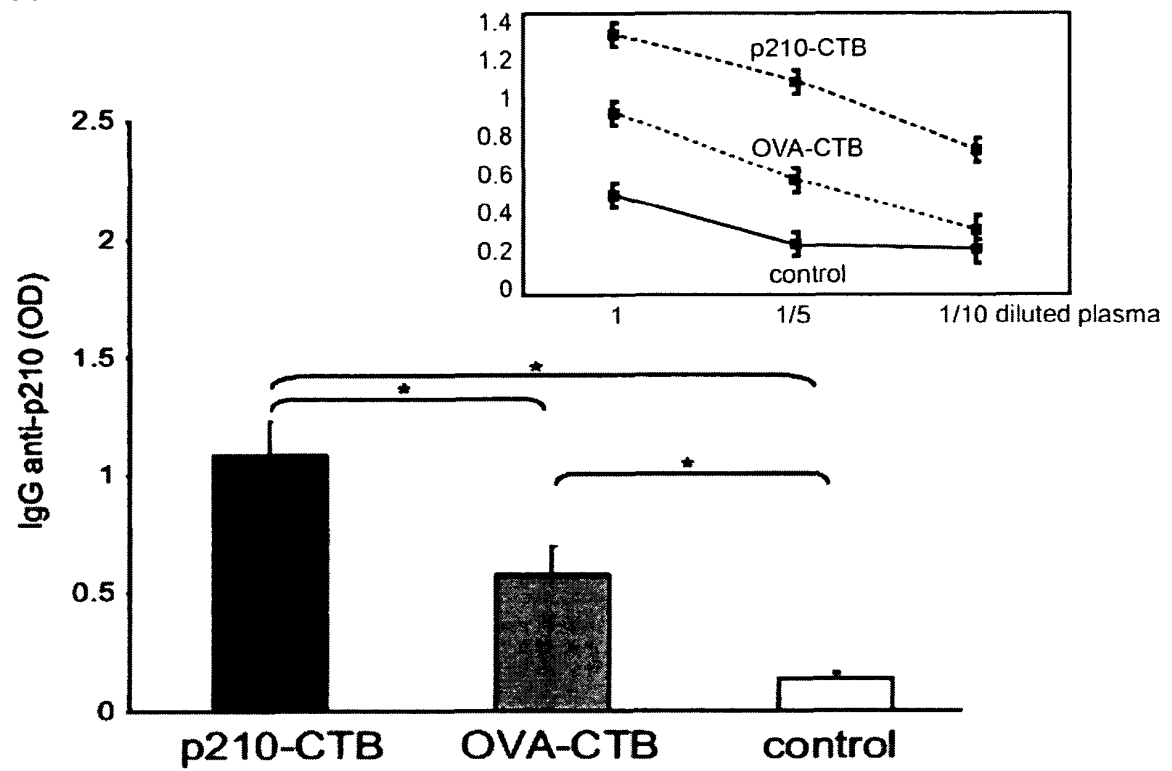
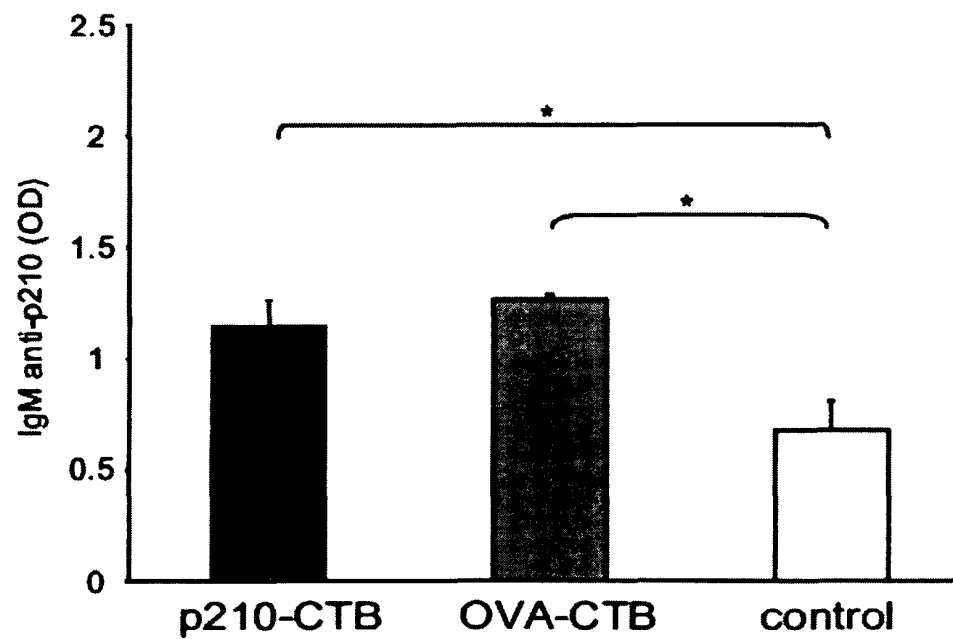
A**B**

FIG. 2

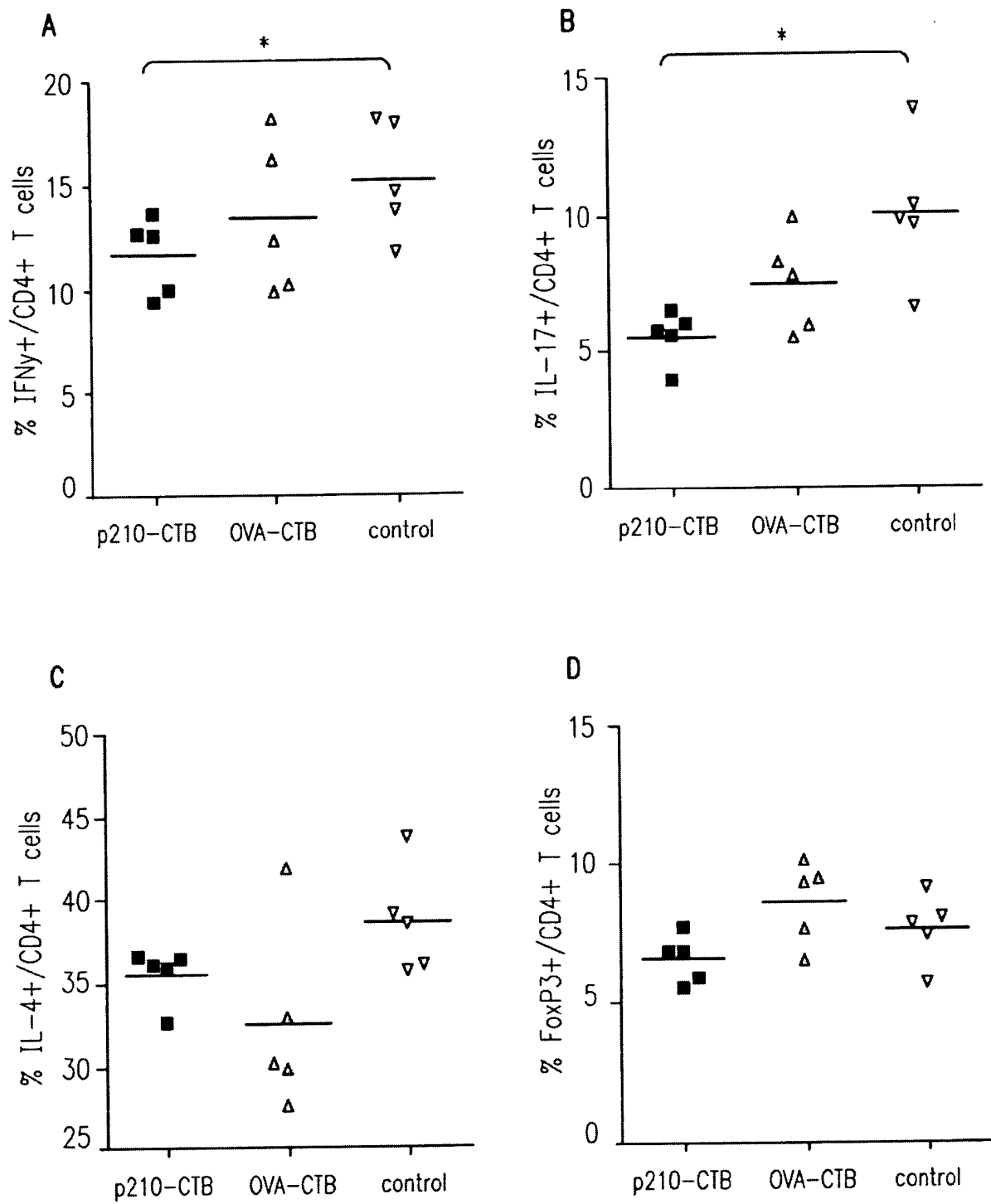


FIG. 3

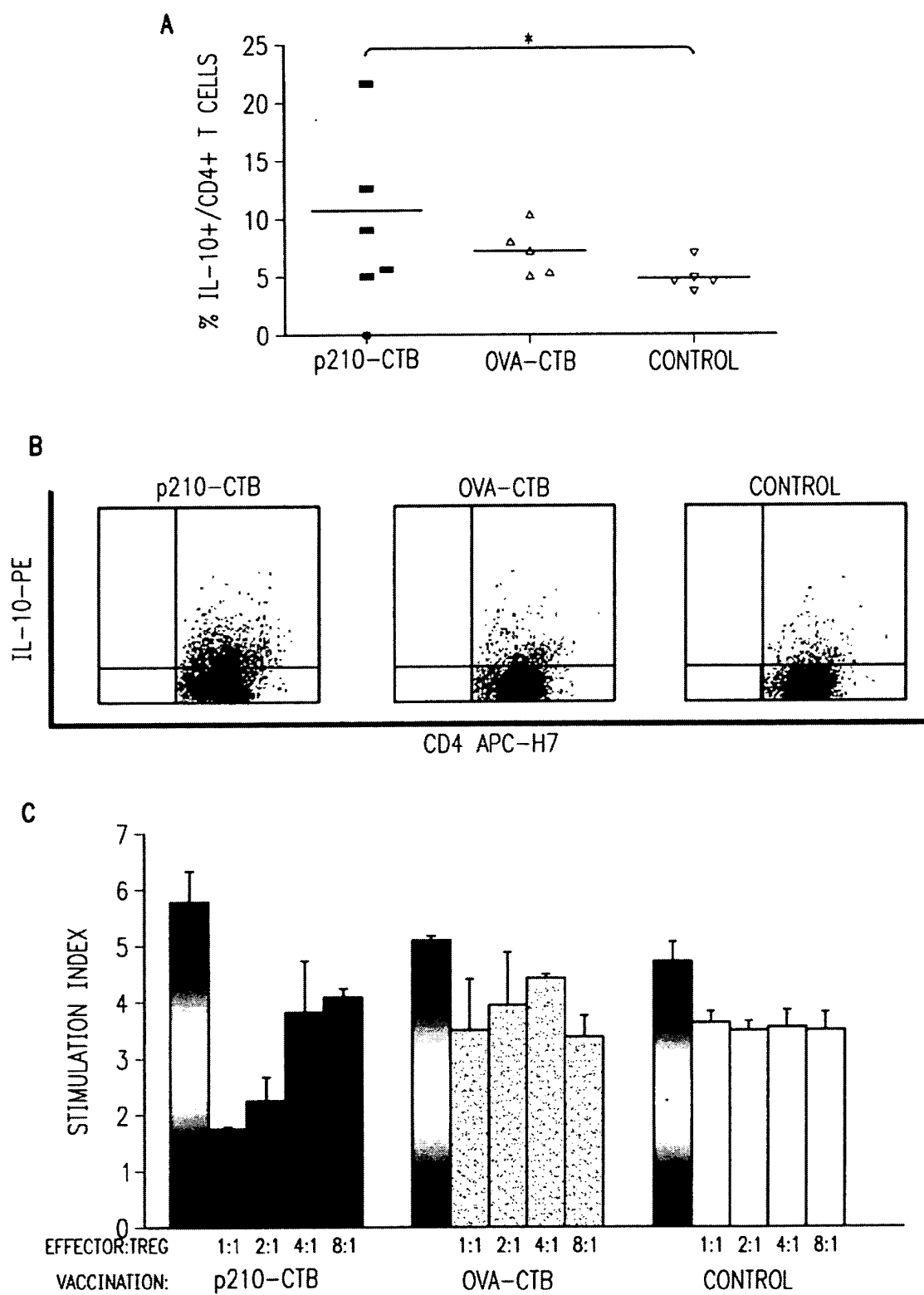


FIG. 4

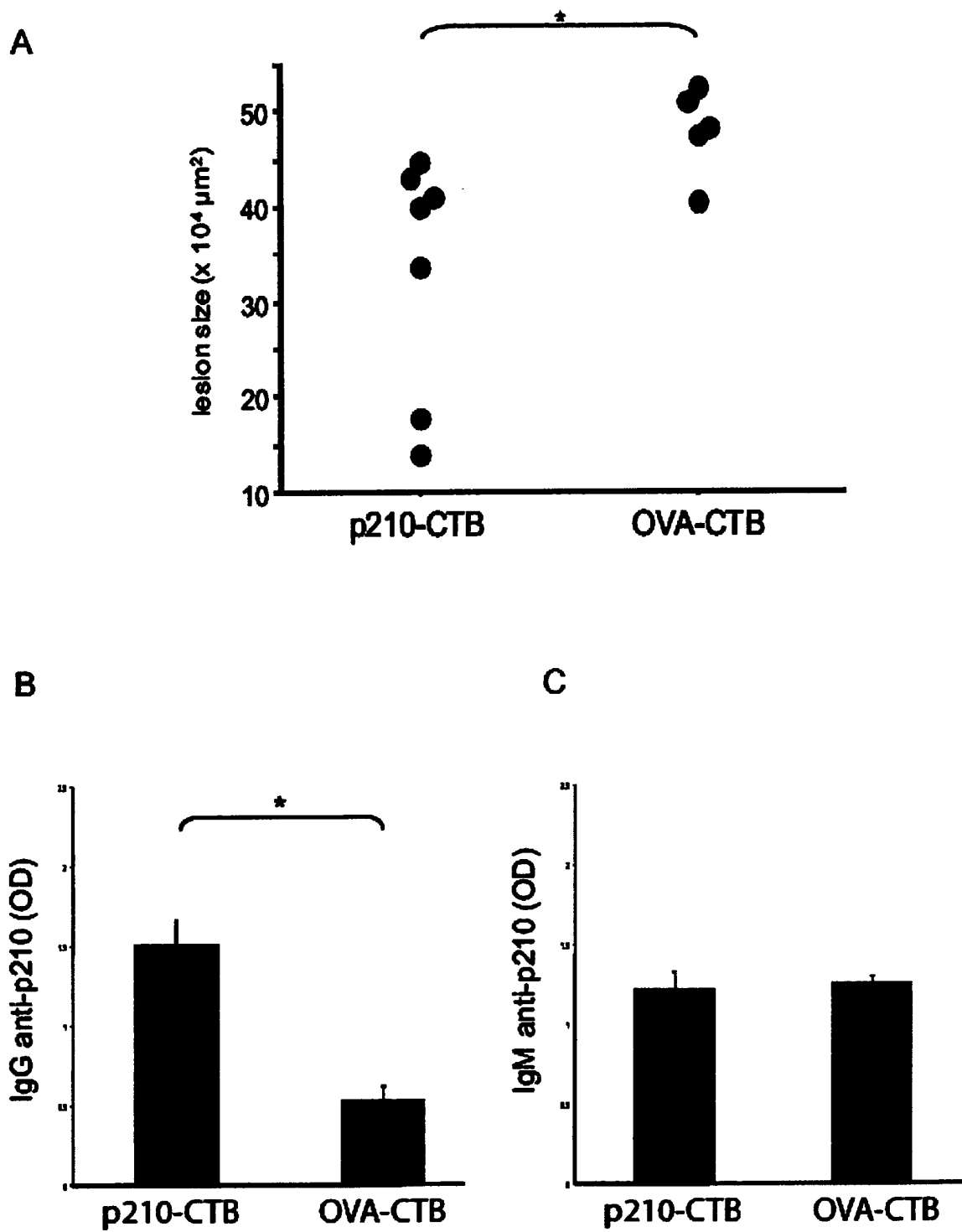


FIG. 5

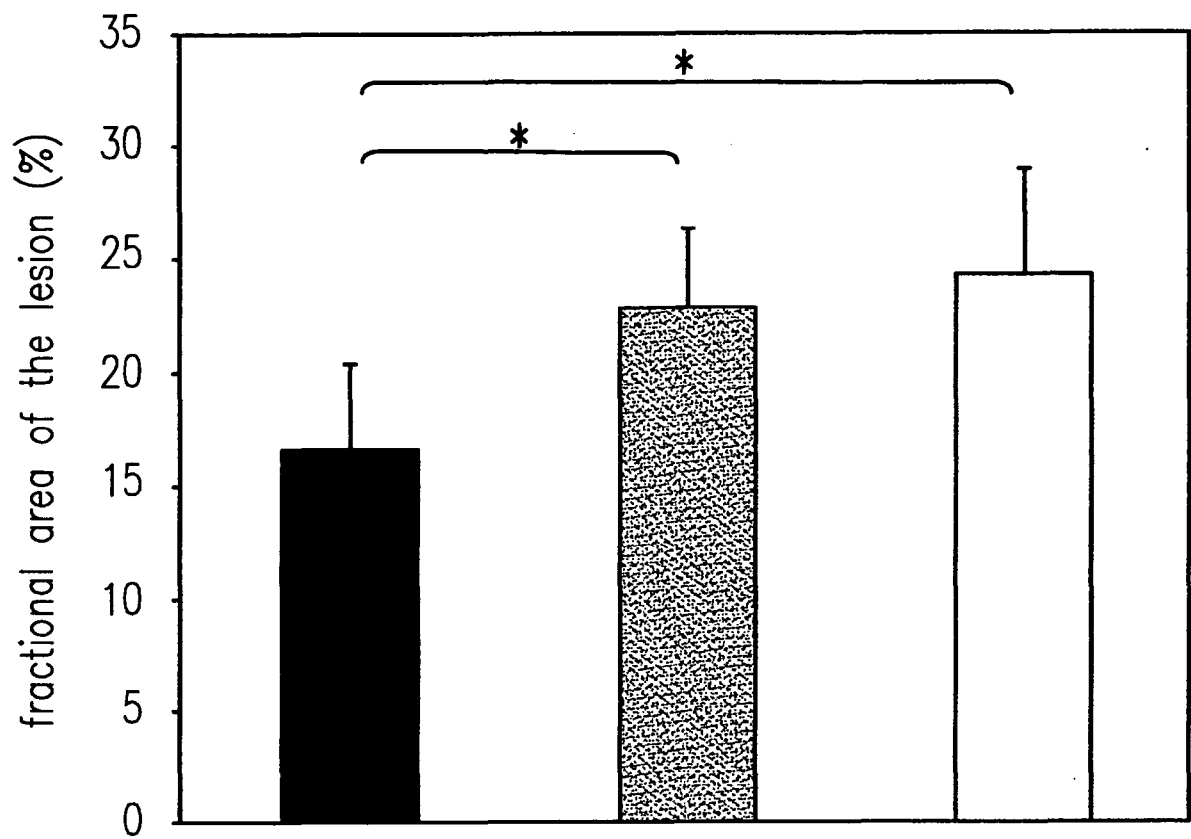


FIG. 6

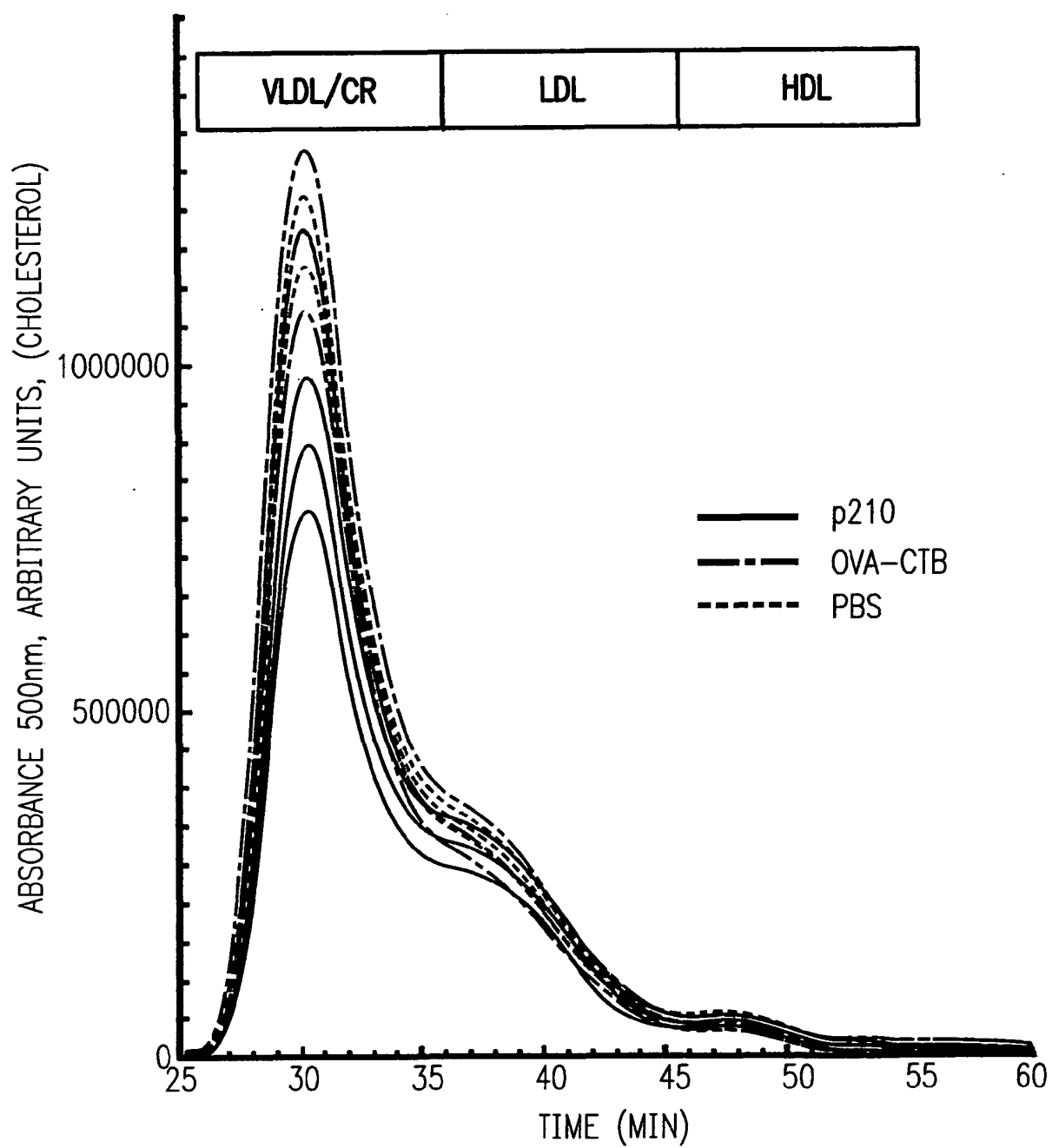
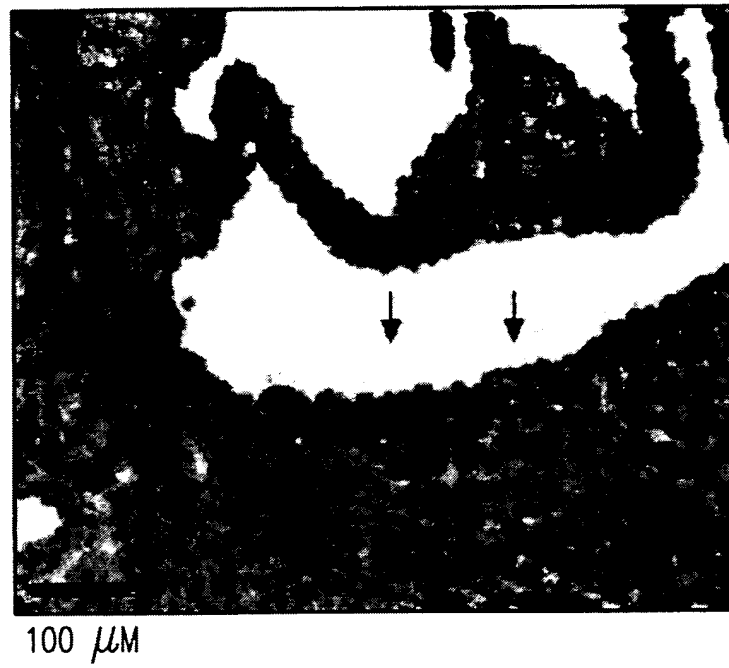


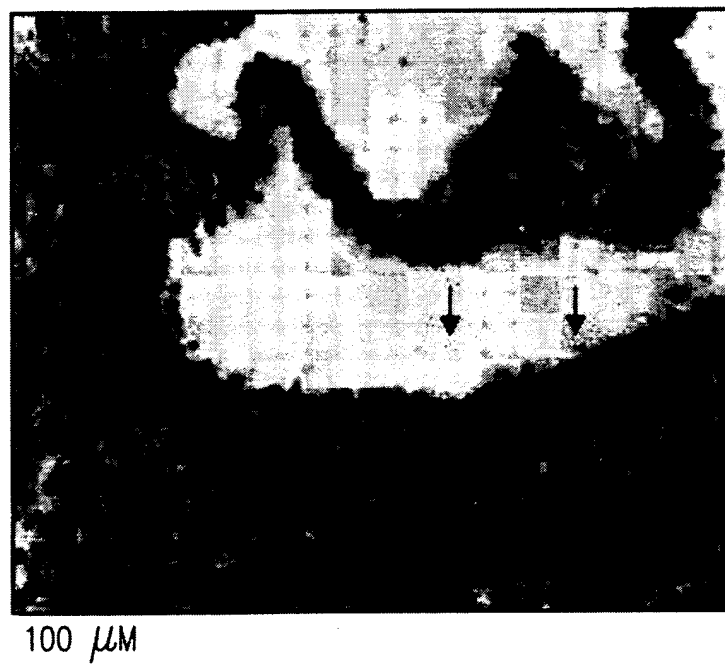
FIG. 7

A



CD4+

B



FoxP3

FIG. 8

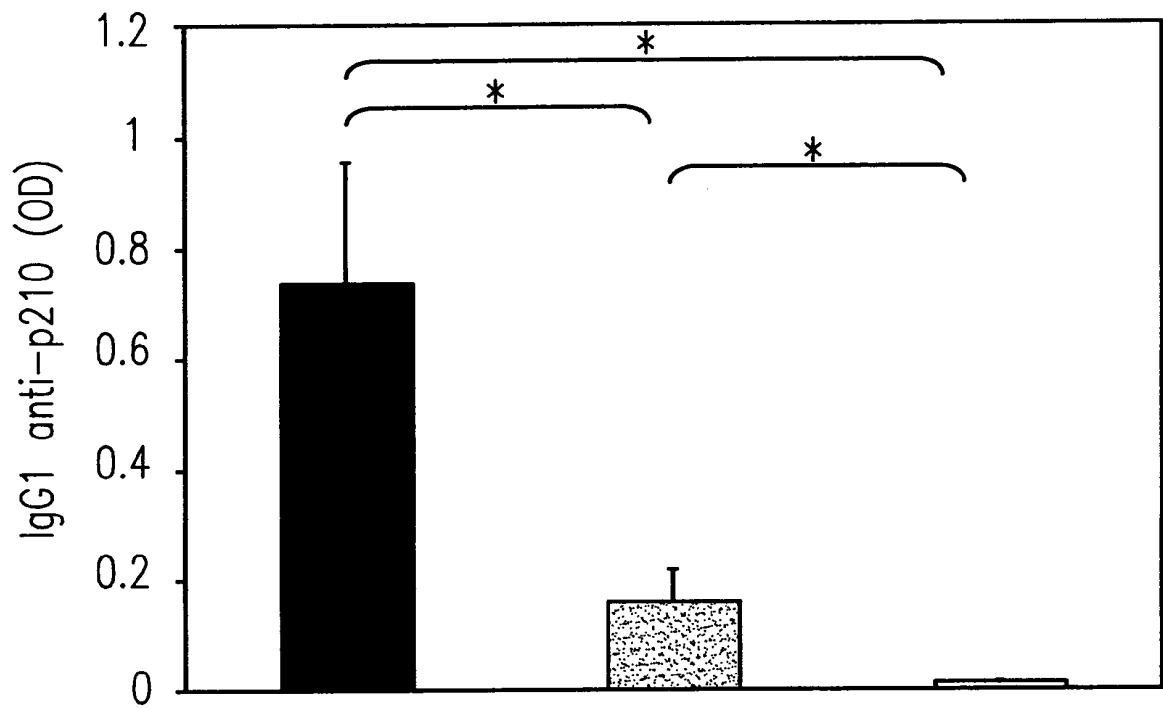


FIG. 9

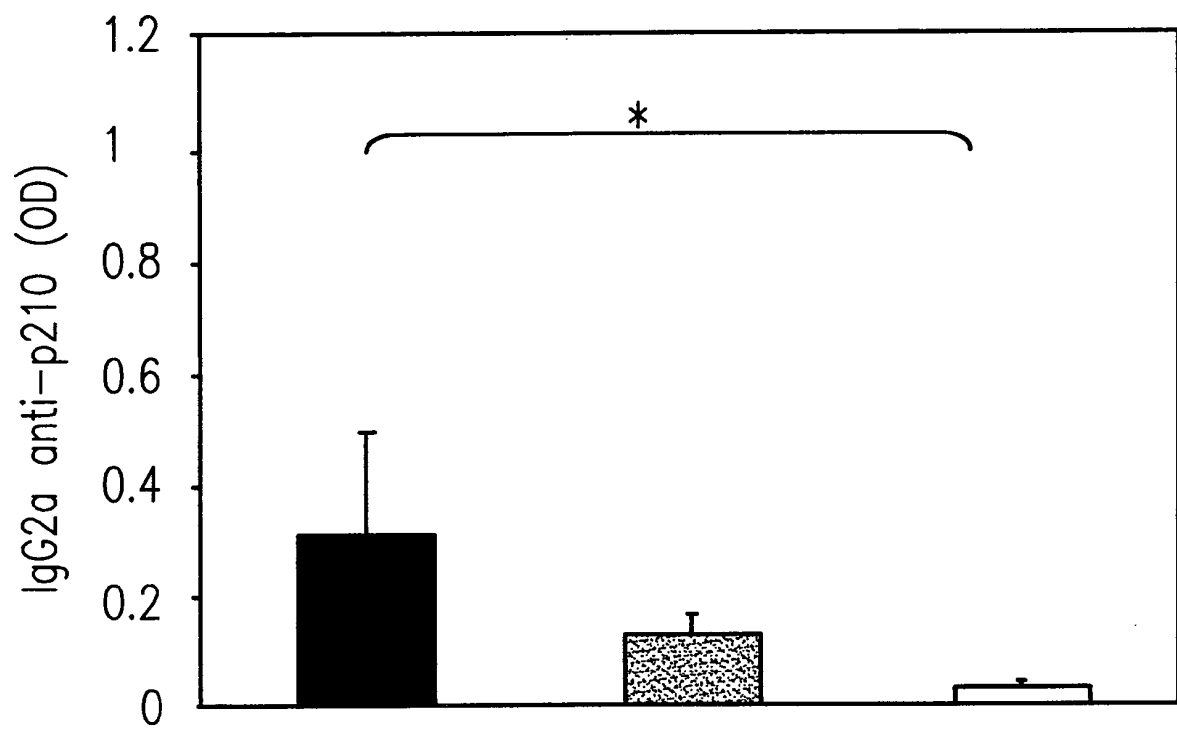


FIG. 10

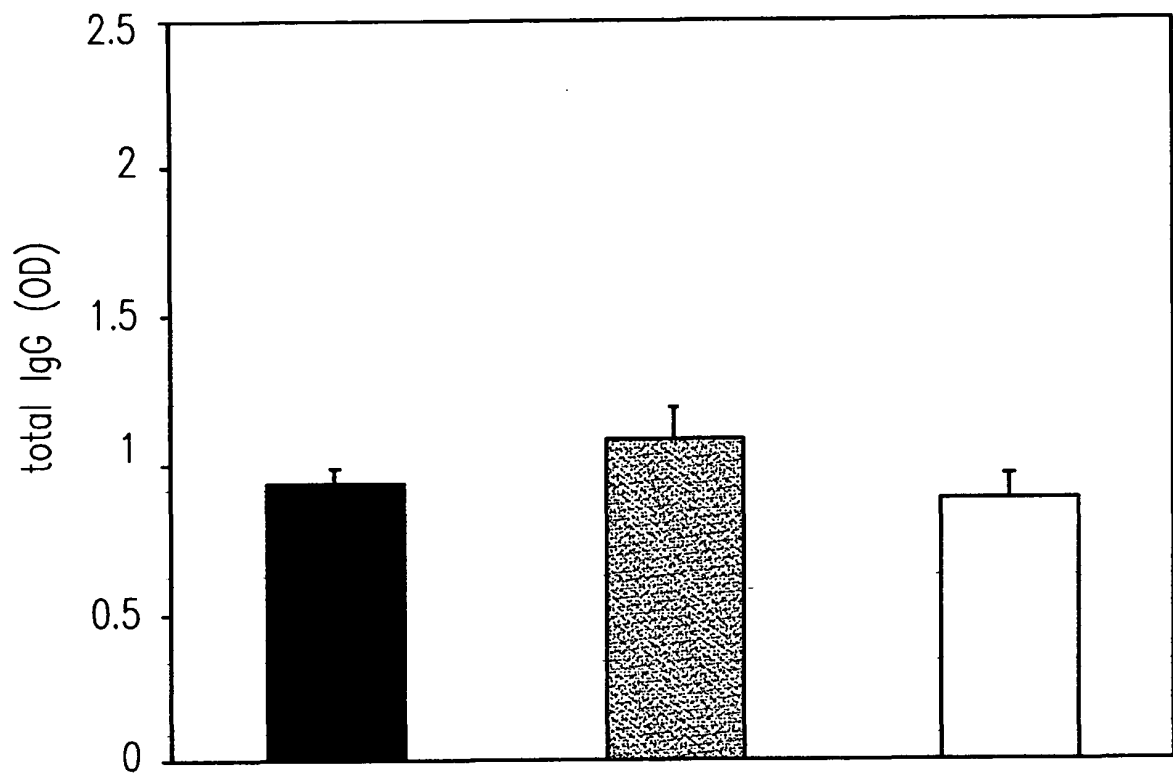


FIG. 11

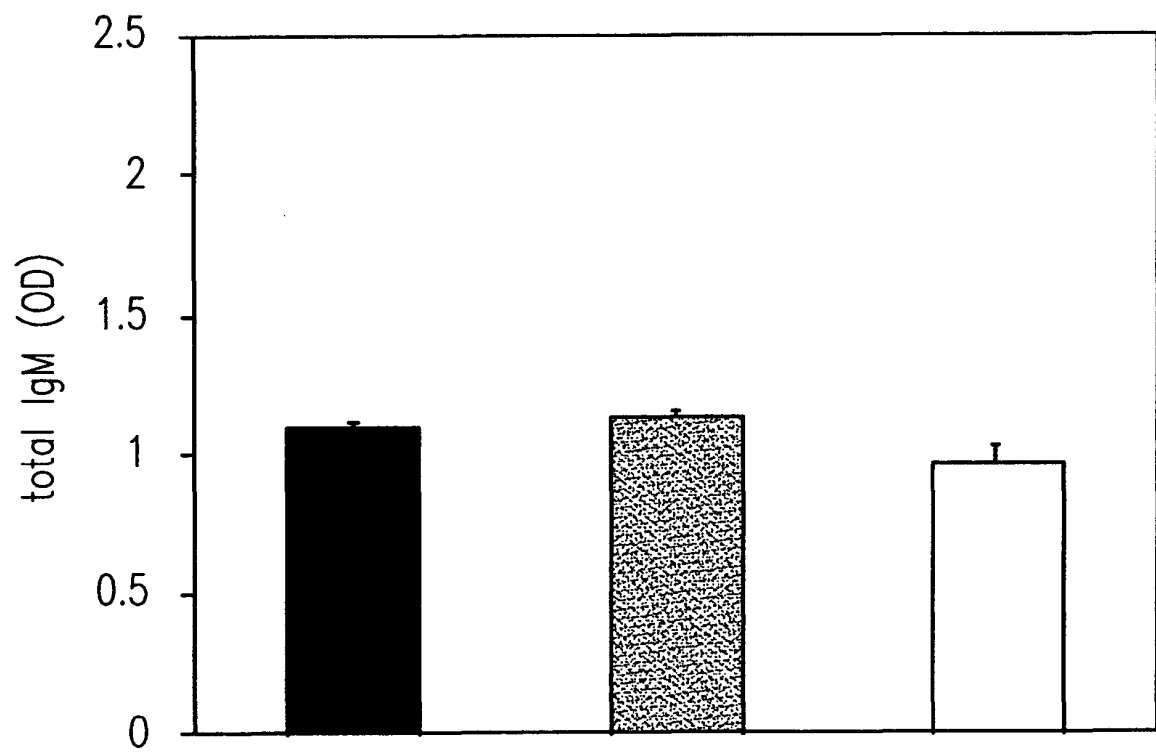


FIG. 12

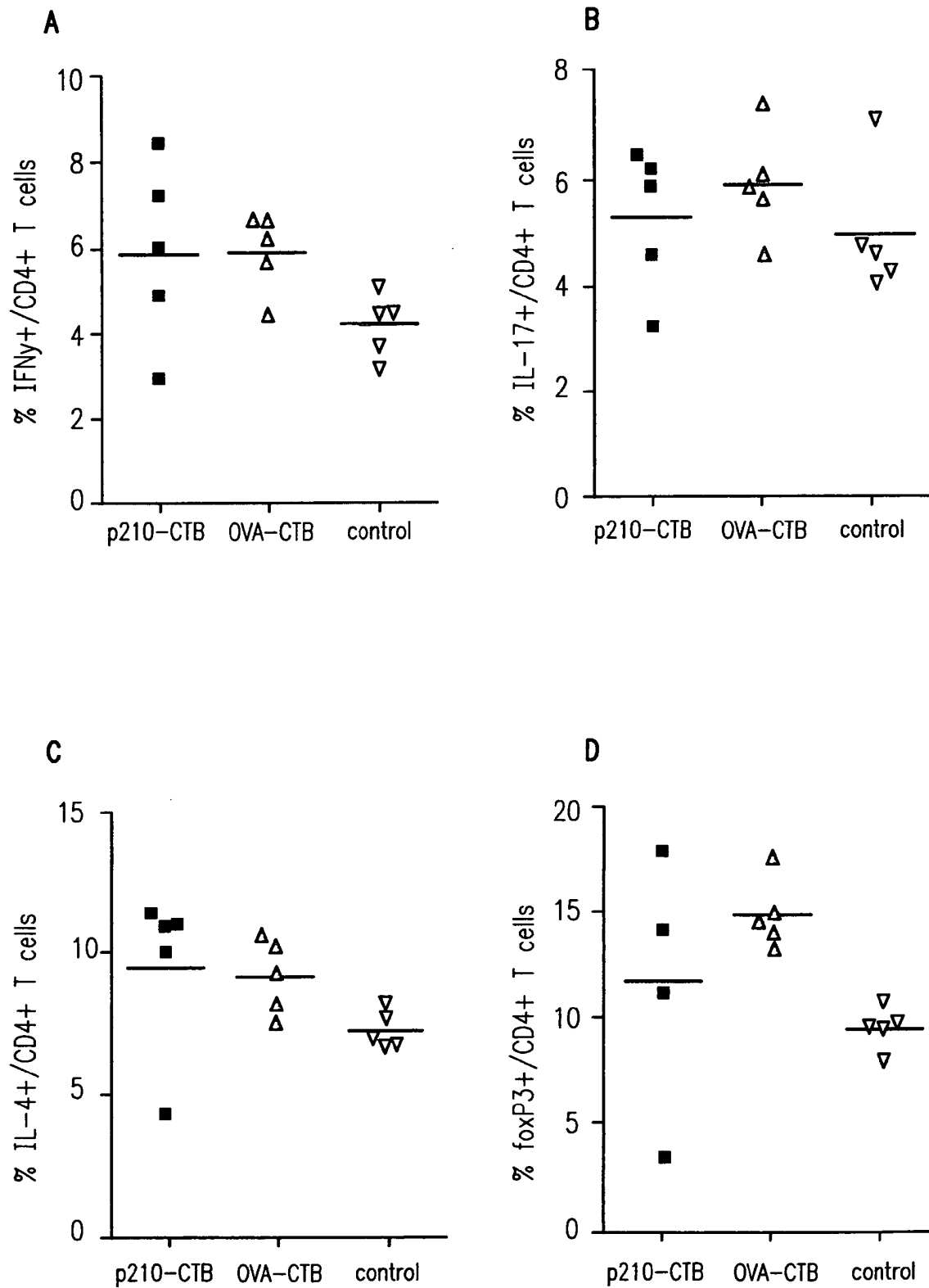


FIG. 13

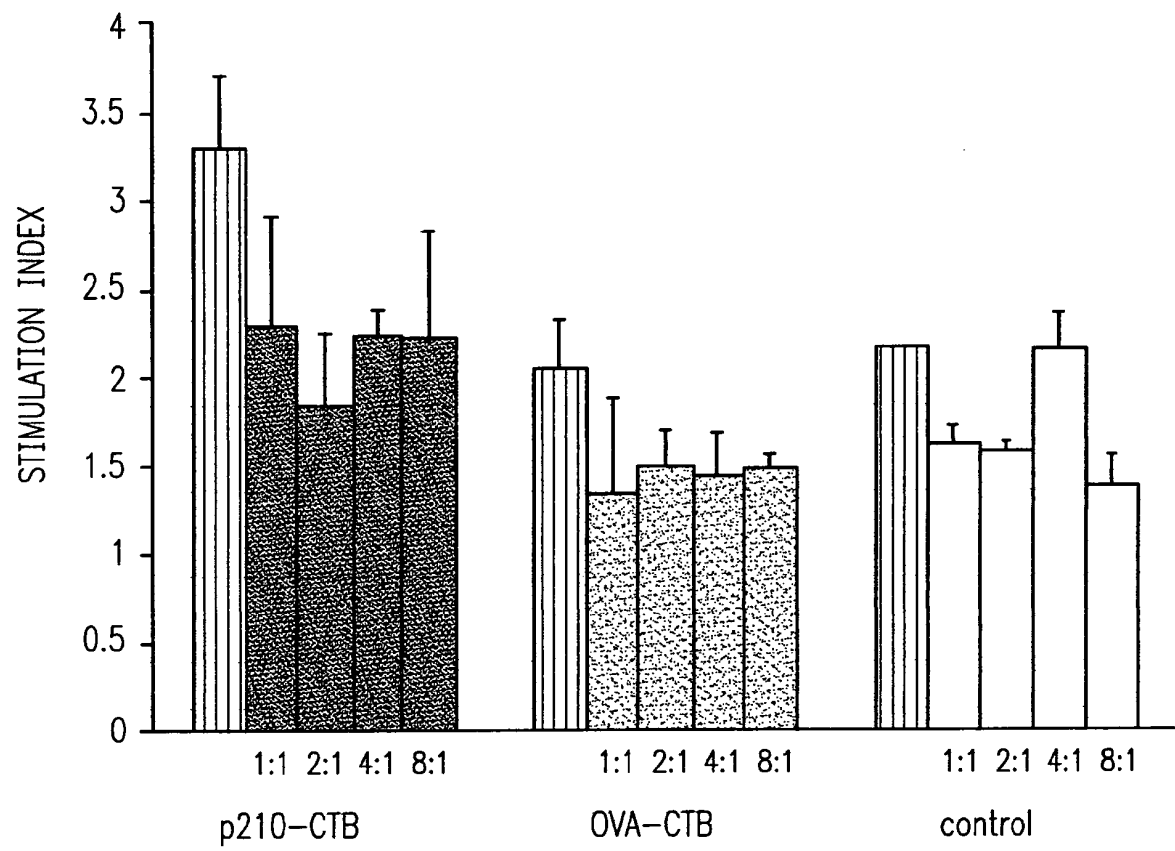


FIG. 14

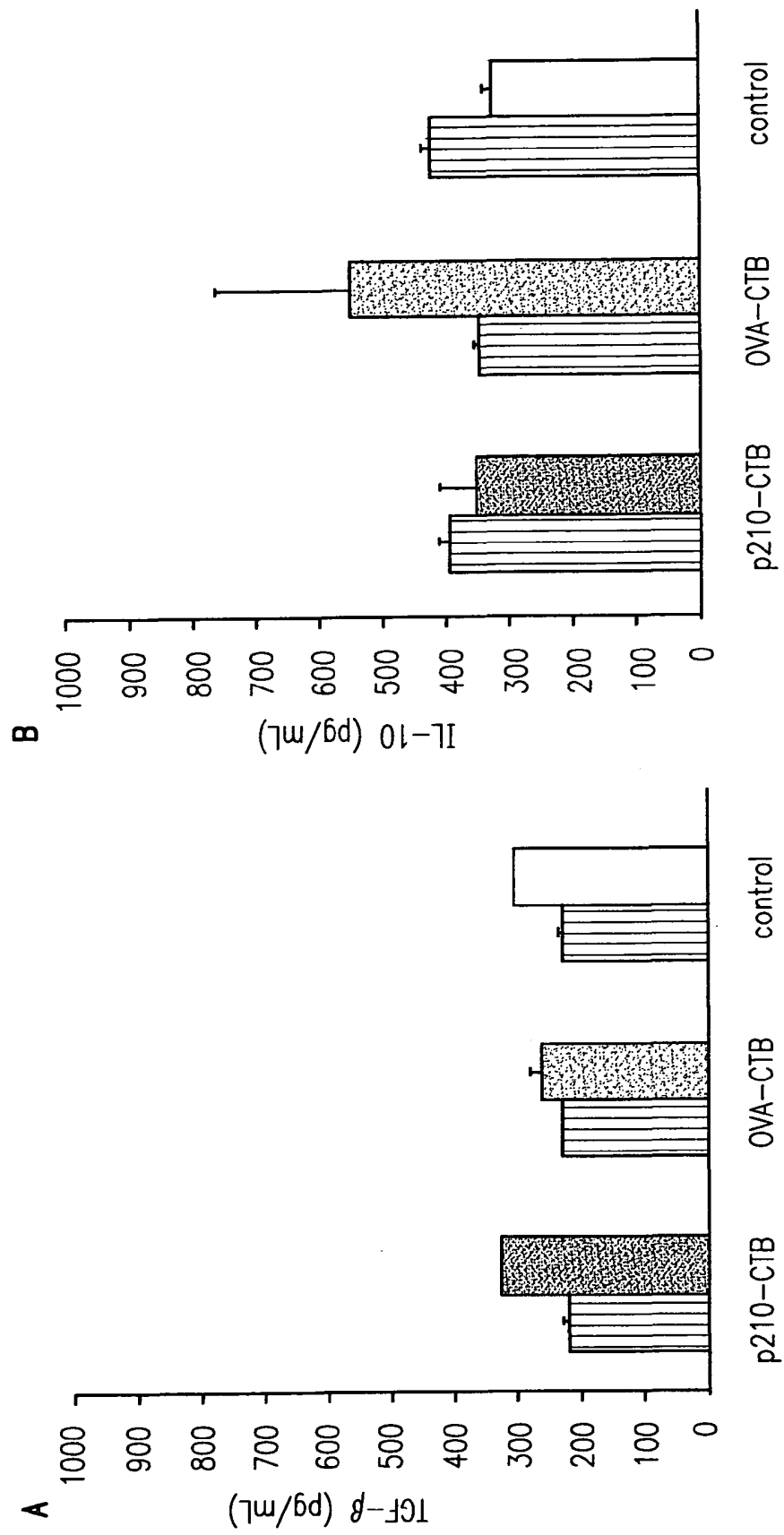


FIG. 15

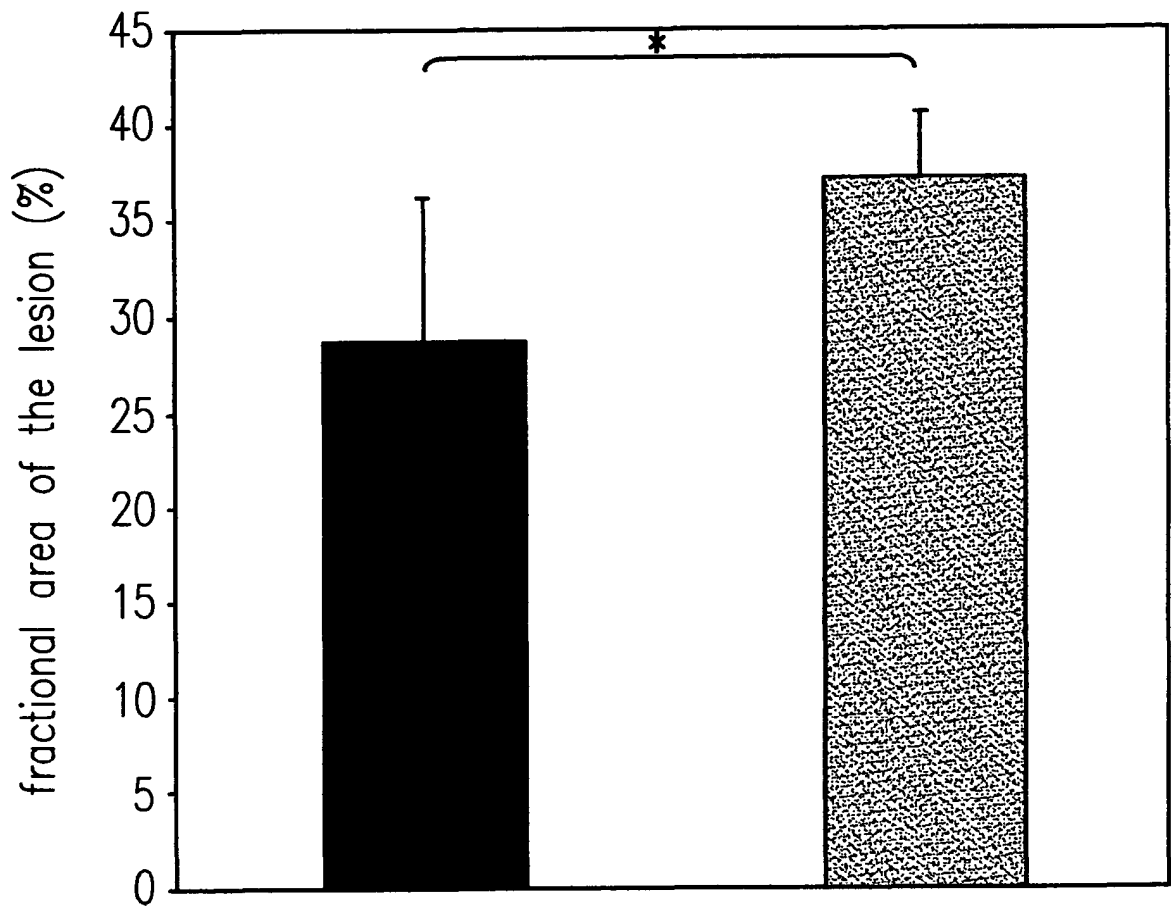


FIG. 16

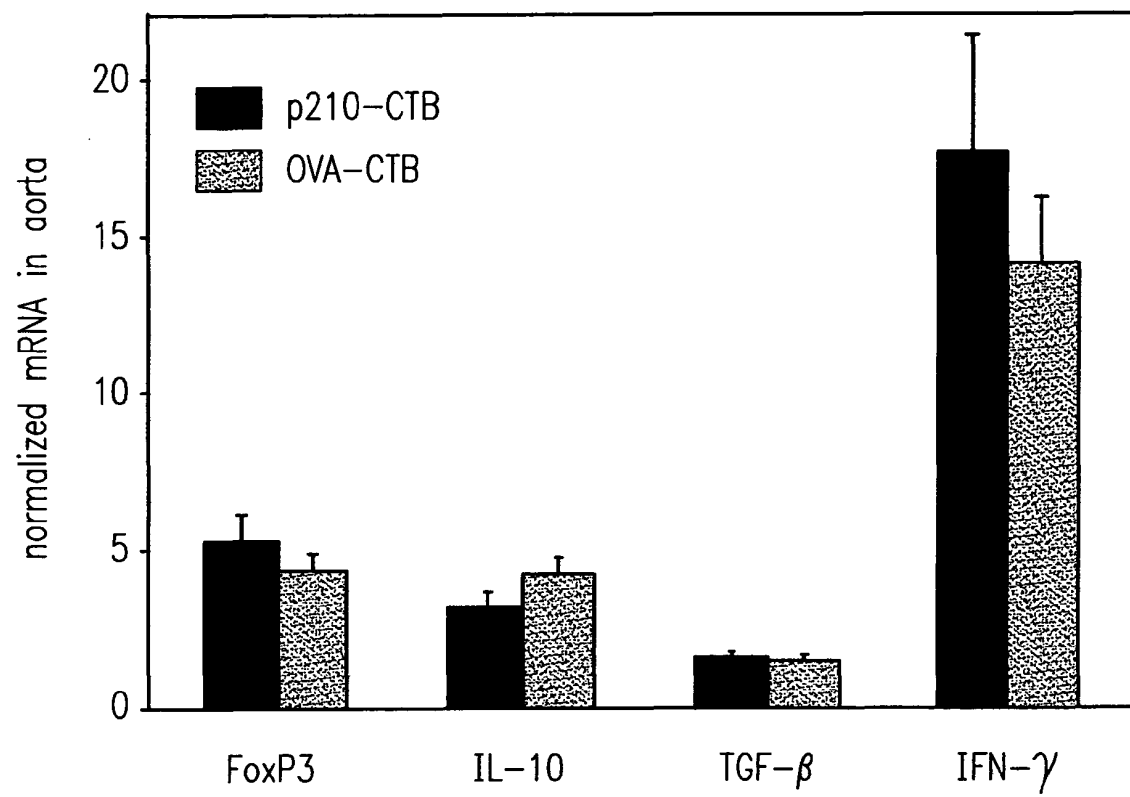


FIG. 17

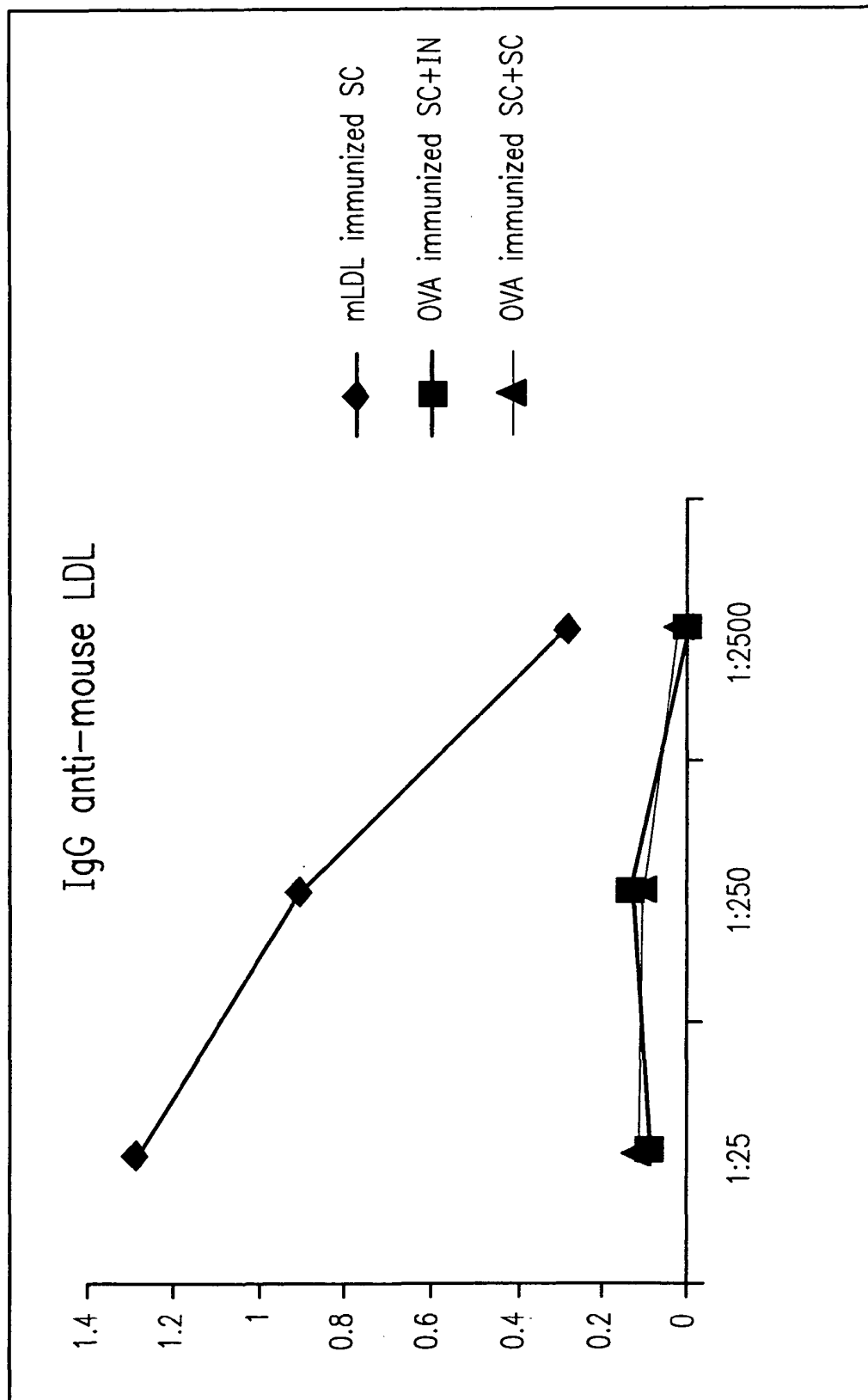


FIG. 18

REFERENCES CITED IN THE DESCRIPTION

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